MOLECULAR PHYSIOLOGY

Biochemistry of blood and regulation of pH

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Blood; Circulation; Red blood cells; Hemoglobin; White blood cells; Platelets; Hemostasis; Homeostasis; Plasma; Buffers; Acid-base balance.
Introduction

Blood, a major part of the milieu interior, is the most important means of communication between the various organs of the body, in addition to being a transport medium for nutrition and excretion. Whereas the nervous system is a means of communication in terms of messages being relayed amongst the organs, physical transportation of raw materials to the various factories of the human body, carrying away the finished products to the site of requirement, and transportation of the waste materials for excretion is achieved by the blood. In addition, blood also carries the basic ingredients for survival to each and every nook and corner – all of this through a properly channelised system!

The blood makes up about 8% of the total body weight of an individual. Thus, in an average person whose body weighs 60 Kg, there is about 4.8 Kg of blood. Being a fluid, it has to have well-defined channels to flow through; these are provided by nature in the form of blood vessels. The blood vessels are a closed tube system (also called vasculature) through which the blood circulates. The exchange of nutrition and excretion is made possible due to varying permeability of the vessel walls in different parts of the body. For example, in the lungs, the vessel walls of the smallest capillaries of the pulmonary vessels are just thin enough to allow a free exchange of gases through them; at the same time, in the kidneys, the permeability of the capillaries which line the Bowman’s capsule allows the passage of solid molecules though it is restricted to the smallest protein molecules. At the other end of the spectrum are the main vessels, the bigger vessels, which are totally impermeable to the passage of blood or its constituents through their walls, thus enabling them to carry the whole blood to its destination without any loss. And, of course, to pump the blood through this channel system we have a specialised pump – the heart.

Circulatory system

The circulatory system comprises of an intricate and graded system of channels that can be classified, for purposes of understanding, into –

1. The arterial system
2. The microcirculation
3. The venous system
4. The lymphatic system.

Arterial system

The arterial system is divided into two main systems – a) systemic which carries oxygenated blood to the capillary beds throughout the body, and b) pulmonary which carries blood to the ‘pulmoner’, or lungs, for oxygenation (Figs 1 and 2). The arterioles, the terminal components of this system, are high-resistance vessels that regulate the distribution of flow to the various capillary beds.

Because of their elasticity, the aorta, the pulmonary artery, and their major branches form a system of channels capable of handling a considerable volume. These two features of the arterial system – its elastic conduits and its high-resistance terminals – are also shared by certain mechanical fluid systems, called hydraulic filters, which tend to dampen fluctuations in flow. Thus, the body’s arterial system constitutes a hydraulic filter; these filters are analogous to the resistance-capacitance filters of electrical circuits.
Fig 1: Heart – Arterial system

Fig 2: Heart – Venous system
The main advantage of hydraulic filtering in the arterial system is that it converts the intermittent output of the heart to a steady flow through the capillaries. The important function of the large elastic arteries has been likened to the Windkessels of antique fire engines. The Windkessels contained a large volume of trapped air. The compressibility of air that remained trapped above the water in the Windkessels converted the intermittent inflow of water from the water source to a steady outflow of water at the nozzle of the fire hose. Without the Windkessel, water would flow only in spurts, making fire fighting inefficient at best and dangerous at worst.

The heart beats about 60-80 times per minute and each phase of contraction followed by relaxation is termed a cardiac cycle. The phase of contraction lasts for only one-third of the cycle; yet blood flows through the vessels continuously.

A small part of the energy of cardiac contraction is dissipated as forward capillary flow during systole; the remainder is stored as potential energy, as much of the stroke volume (the amount of blood pumped out by the heart during one cardiac cycle) is retained by the distensible arteries (Fig. 4). During diastole (the relaxation phase of the cardiac cycle), the elastic recoil of the arterial walls converts the potential energy into capillary blood flow. If the arterial walls were rigid, as happens in certain pathological conditions like arteriosclerosis (where there is calcification and changes in content of collagen and elastin in arterial walls), capillary flow would not occur during diastole.
Pumping in spurts requires more energy than pumping in a steady flow. In addition, this energy requirement is modified by variations in heart rate, peripheral resistance and arterial distensibility, whatever the situation, the hydraulic filtering minimizes this work of the heart and hence improves its efficiency.

This is reflected by experiments in representative animals which show that for any given stroke volume, passing the blood through a plastic tube (rigid) instead of the aorta, increases the oxygen consumption of the heart, indicating an increased amount of work done. It is evident here that this intermittent pushing of blood into the arterial system will have a similar effect on the pressure in these vessel/chambers. Hence, the blood pressure has systolic and diastolic levels that indicate the highest and the lowest pressures, respectively of the range of pressure exhibited by the fluid blood.

As the blood flows from the bigger arteries into the smaller arterioles, it obviously is restricted by the decreasing diameter of the channel. This is termed as the peripheral resistance and this, too, modulates the blood pressure. At the same time, all the blood ejected by the heart does not, therefore, flow into smaller vessels with that stroke; there is some volume left behind which accounts for the apparently continuous blood flow into the arterial system.

**Microcirculation**

The arterial system delivers blood through the arteries and arterioles (10-20 µm diameters) to every part of the body without loss. But in the tissues, the blood must deliver its gases and nutrients – the major purpose for which it traverses the distance of the blood vessels. Hence, from the arterioles, the blood flows into the capillaries whose walls are semi-permeable, or even selectively permeable at places.

The capillary walls consists of a single layer of epithelial cells which permit the rapid exchange of gases, water and solutes with the interstitial fluid. The channel system is such
that blood flows from an arteriole to a bed of capillaries and adjacent capillary beds (or networks) communicate with each other so that the capillary bed in one area of the body becomes one large network of blood delivery to that organ or part

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This network of capillaries is filled, on one hand, by the blood from the arterioles and metarterioles (5-10 µm diameters) and empties into a corresponding network of venules (Fig. 5).

![Fig.5: Microcirculation](image)

This channel system comprised of the arterioles, the capillaries and the venules constitutes the microcirculation.

**Venous system**

As the blood flows to each part of the body, so must it return to be re-circulated (see Figs 1 and 2). The channel system through which it returns to the heart corresponds to the delivery system so that for almost every arteriole there is a venule and for almost every artery there is a vein. This system of channels, called the venous system, consists of veins through which blood flows passively. To aid this passive movement, the venous system has valves, which do not allow the reverse flow of blood that can occur due to gravity or an injury in which the proximal vein gets emptied.

In addition, peripheral muscle activity promotes the flow of blood through the veins towards the heart.
**Lymphatic system**

As the blood flows through the capillaries, and as the exchanges of gases, water and solutes proceeds some solutes, especially proteins, which are not required by the tissue and a lot of water along with them, also get transferred out of the capillaries. To recapture these substances, nature has devised yet another system of channels, called the lymphatics, which drain the interstitial fluid and carry it to the veins, thus transporting all the solutes and water, not required in the periphery, back into the circulation.

The lymph capillaries differ from the blood capillaries in that they are anchored to the surrounding connective tissue by fine filaments. These filaments have specific function in that, with muscular contraction, these strands pull at the lymphatic vessels and open up the spaces between the endothelial cells lining the channel. These spaces permit the entrance of protein and large particles into the lymphatic vessels. The lymph capillaries drain into larger vessels that finally enter the right and left subclavian veins where they connect with the respective internal jugular veins.

In addition to returning fluid and protein to the circulation, the lymphatic system filters the lymph at the lymph nodes and removes foreign particles, such as bacteria. The largest lymphatic vessel, the thoracic duct, not only drains the lower extremities, but it also returns proteins lost through the permeable liver capillaries. The thoracic duct also carries substances absorbed from the gastrointestinal tract, principally fat in the form of chylomicrons. Thus, we find that lymph is a clear-to-white fluid made of:
- Chyle i.e. fats and proteins (from the interstitial fluid as well as from the gastrointestinal tract)
- Some red blood cells
- Many white blood cells, especially lymphocytes (to combat bacteria).

**Biomedical aspects**

1. When either the volume of interstitial fluid exceeds the drainage capacity of the lymph vessels, or there is a blockage in these vessels, interstitial fluid accumulates. Such accumulation occurs directly in the more compliant tissues (e.g. subcutaneous tissue), and it gives rise to clinical oedema.
2. In many infections, the lymph nodes are seen to be enlarged due to a large volume not only of bacteria, but also the inflammatory response of the body by which leucocytes, the fighters of the body, rush to the site of high bacterial content (e.g. tonsillitis, inflammation of the adenoids, lymph adenitis).

There are some parts of the body, where the circulation has some special features. These are:

**Systemic and cardiopulmonary circulations**

Most arteries carry oxygenated blood rich in nutrients from the heart to the various systems of the body (essentially to every part of the body) and most veins drain the deoxygenated blood from these systems and bring it all back to the heart. This circulation is called the systemic circulation.
In addition to this, there is the cardio-pulmonary circulation, where the pulmonary artery (unlike other arteries) carries deoxygenated blood to the lungs where gaseous exchange with the inhaled air occurs, and then the pulmonary vein (unlike other veins) carries oxygenated blood to the heart for subsequent transport through the systemic circulation (see Figs 1, 2 and 3). The process is a continuously repeating one.

As the heart beats, the left ventricle of the heart pumps oxygenated blood into the systemic circulation and, at the same time, the right ventricle of the heart pumps deoxygenated blood into the pulmonary circulation.

The venae cava, which are the largest veins returning the blood from the systemic circulation, drain into the right atrium which lets blood into the right ventricle during diastole whence it proceeds to the pulmonary circulation through the pulmonary artery during systole. The pulmonary vein brings back oxygenated blood from the lungs and empties it into the left atrium whence blood is let into the left ventricle and thence pumped into the systemic circulation.

**Coronary circulation**

The right and the left coronary arteries arise at the root of the aorta behind the right and the left cusps of the aortic valve, respectively. Theses arteries provide the entire blood supply to the myocardium, the respective arteries supplying the respective sides of the heart. The left coronary branches into the anterior descending and the circumflex branches. After the coronary arterial blood has passed through the capillary beds, most of it returns to the right atrium through the coronary sinus, but some of the coronary venous blood reaches the right atrium by way of the anterior coronary veins.

In addition, vascular communications link the vessels of the myocardium and the cardiac chambers; these communications are:
1) Arteriosinusoidal vessel
2) Arterioluminal vessel
3) Thebesian vessel

**Arteriosinusoidal** Channels consist of small arteries or arterioles that lose their arterial structure as they penetrate the chamber walls, where they divide into irregular endothelium-lined sinuses, which anastomose with other sinuses and the capillary bed.

**The arterioluminal vessels** are small arteries or arterioles that open directly into the atria and ventricles.

**The Thebesian vessels** are small veins that connect capillary beds directly with the cardiac chambers and that also communicate with cardiac veins.

All the minute vessels of the myocardium communicate in the form of an extensive plexus of subendocardial vessels.

**Metabolic considerations**

A striking characteristic of the coronary circulation is the close relationship between the level of myocardial metabolic activity and the magnitude of coronary blood flow. The
mechanism that links them remains unsettled. However, it appears that a decrease in the ratio of oxygen supply to oxygen demand releases a vasodilator substance (e.g. adenosine) from the myocardial cells into the interstitial fluid, where it relaxes the coronary resistance vessels (Fig 6).

![Myocardial oxygen balance diagram]

Fig 6: Myocardial oxygen balance

Numerous agents mediate the vasodilation that accompanies increased cardiac work, e.g. adenosine, which induces vasodilation by activating adenosine receptors in the coronary resistance vessels.

Accumulation of the vasoactive metabolites may also be responsible for reactive hyperemia because the duration of the enhanced coronary flow after release of the briefly occluded vessel is, within certain limits, proportional to the duration of the period of occlusion. Among the factors implicated are CO₂, hydrogen ions, potassium ions, hypoxia and adenosine.

**Biomedical aspects**

**Myocardial stunning** is a pronounced mechanical dysfunction caused by a relatively brief period of severe ischaemia followed by reperfusion. What makes the myocardium so susceptible is that even when the coronary blood flow is normal, the extraction of oxygen from each unit volume of blood is nearly maximal. Hence, even a small decrease in flow (which may be neither too prolonged nor too severe) can still cause myocardial hypoxia and thence, dysfunction.

If the myocardial flow does not return to normal soon enough, the person is said to have had a heart attack [*myocardial infarction*], which is gross impairment in the electrical and mechanical behavior of the heart.

**Cutaneous circulation**

The oxygen and nutrient requirements of the skin are relatively small. Hence, in contrast to most other body tissues, the supply of oxygen and nutrients is not the chief factor in the regulation of cutaneous blood flow.
The primary function of the cutaneous circulation is the maintenance of a constant body temperature. Consequently, the skin undergoes wide fluctuations in blood flow depending on whether the body needs to lose or conserve heat. Mechanisms responsible for alterations in skin blood flow are mainly activated by changes in ambient and internal body temperatures. Most of this regulation is through the nervous systems (central as well as autonomous); however, there is one biochemical mechanism, which deserves mention here. Sweat contains an enzyme that acts on a protein-moiety in the tissue fluid to produce bradykinin, a polypeptide with potent vasodilator properties. It dilates the arterioles thereby increasing blood flow to the skin and, thence, heat loss from the skin.

**Biomedical aspects**

When the body temperature is raised, as in fever (of any origin), one of the most evident mechanisms for reduction of this temperature is a promotion of sweating all over the body which, via the bradykinin, helps to lower the temperature. Many of the antipyretics prescribed (e.g acetaminophen, acetylsalicylic acid, etc.) actually bring down the temperature by stimulating this mechanism via the central nervous system.

**Cerebral circulation**

A unique feature of the cerebral circulation is that it lies within a rigid structure, the cranium. Since intracranial contents are incompressible, any increase in the arterial inflow, as induced by arteriolar dilation must be associated with a comparative increase in venous out-flow. The volume of blood and of extravascular fluid can vary considerably in most body tissues. In the brain, however, the volume of blood and extravascular fluid is relatively constant; a change in one of these fluid volumes must be accompanied by a reciprocal change in the other.

**Biomedical aspects**

When anyone suffers a stroke (unilateral paralysis) due to rupture of an intracranial vessel, or if a person suffers a head injury where there is damage to an intracranial vessel, the accumulation of blood that has flown out of the circulatory system causes an increase in intracranial pressure, resulting in concussion. It may even cause defects that can be attributed to pressure on areas of the brain corresponding to the areas of the defect. For e.g. if there is pressure on the Broca’s area (the area responsible for language processing and speech production), the person’s speech will be affected.

**Intestinal circulation**

The microcirculation in the intestinal villi is such that counter current exchange system is established so that $O_2$ from the arterioles may diffuse directly into the venules. This places the villi in jeopardy in states of low blood flow.
**Hepatic circulation**

The liver has a dual blood supply; three-fourth comes from the portal vein, which drains the whole Gastro-intestinal tract, and one-fourth comes from the hepatic artery. Since the portal venous blood has already traversed the Gastro-intestinal tract, it has been deprived of its oxygen content hence three-fourths of the oxygen supply to the liver comes from the hepatic artery, whereas only one-fourth comes from the portal vein.

**Fetal circulation**

Before birth, the circulation of the fetus differs from that of the postnatal infant. The most important difference is that the fetal lungs are functionally inactive, and the fetus depends entirely on the placenta for oxygen and nutrient supply.

Since the lungs are not functional, inasmuch as gaseous exchange is concerned, a large percentage of the right atrial blood passes through the foramen ovale to the left atrium, and a large percentage of the pulmonary arterial blood passes through the ductus arteriosus to the aorta. The reduction of pulmonary vascular resistance caused by lung inflation at the time of birth is the main factor that reverses the pressure gradient between the atria, and thereby closes the foramen ovale. A similar alteration in pressure gradient between the pulmonary and the aorta, albeit of a smaller magnitude, aids the closure of the ductus arteriosus.

![Fetal circulation diagram](image)

**Fig 7: Fetal circulation**
**Biomedical aspects**

Sometimes, the reversal of the pressure gradient between the atria is insufficient for the closure of the foramen ovale, which persists in the child after birth and causes mixing of the blood of the two atria. The result is that the blood that is pumped out of the heart to the rest of the body is not fully oxygenated and the delivery of oxygen is less than it should be. This is evidenced as cyanosis – a dusky discoloration of the skin due to the presence of deoxygenated blood in the capillary beds. The ductus arteriosus can remain patent for a few weeks after birth, but rarely this patency persists, again resulting in deficient oxygen delivery to the tissues. For both these situations surgical correction is required.

**Formation of blood (Hemopoiesis)**

The blood is composed of various cellular elements suspended in a fluid called plasma. These cellular elements are produced by a regulated process called hemopoiesis (Fig 8).

The cells are of three types – the red blood cells or red blood corpuscles (RBC or erythrocytes), the white blood cell or white blood corpuscles (WBC or leucocytes) and the platelets (thrombocytes). The white blood cells are again classified into three categories – the granulocytes, monocytes and lymphocytes.

**Table 1: Life span of various blood cell types**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Average Life Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cells (7-9µm)</td>
<td>120 days</td>
</tr>
<tr>
<td>White cells – Granulocyte</td>
<td>Average: 11-16 days</td>
</tr>
<tr>
<td>Neutrophils (10-12 µm)</td>
<td>1-4 days</td>
</tr>
<tr>
<td>Basophils (10-12 µm)</td>
<td>8-12 days</td>
</tr>
<tr>
<td>Eosinophils (10-15 µm)</td>
<td>8-12 days</td>
</tr>
<tr>
<td>Monocytes (14-20 µm)</td>
<td>Few months – few years</td>
</tr>
<tr>
<td>Lymphocytes (sm=6-8 µm; l=13-20 µm)</td>
<td>Several years</td>
</tr>
<tr>
<td>Platelets (2-4 µm)</td>
<td>7-10 days</td>
</tr>
</tbody>
</table>

Most blood cells have a relatively short life span (Table 1) and their numbers are maintained by continuous replenishment from stem cells, which are the parent cells. A stem cell must have the capability of self-renewal in order to maintain the stem cell pool, and must also have the capability to differentiate into mature cells of various cell types – hence, the name pleuripotent myeloid stem cell (PMSC) – which may give rise to the progenitors of the erythroid, megakaryocytic and the granulocytic cell lines (Fig. 4). These progenitor cells are usually designated by the suffix “-poietic”

Normal hemopoiesis in the adult depends on the following factors:
- the production of blood cells from their recognizable precursors in the bone marrow,
- their survival in the vasculature, and
- their demise in the reticuloendothelial system (RES), predominantly the spleen, liver lung and the marrow itself.
Though the concentration of these cells in the blood varies widely, the values observed in normal individuals are remarkably consistent (Tables 2 and 3), particularly considering the vast differences in the life spans of these cells.

Table 2: Biological reference interval (normal values) for the red cell series in adults

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RBC Count</td>
<td>M: (5±0.5) x 10^{12}/L; F: (4.3±0.5) x 10^{12}/L</td>
</tr>
<tr>
<td>Haemoglobin (of whole blood)</td>
<td>M: 15.0±2.0 gm/dL; F: 13.5±1.5 gm/dL</td>
</tr>
<tr>
<td>Haematocrit / PCV (packed cell volume)</td>
<td>M: 45±5%; F: 41±5%</td>
</tr>
<tr>
<td>Mean Corpuscular volume (MCV)</td>
<td>92 ± 9 fl</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin (MCH)</td>
<td>29.5 ± 2.5 pg</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration (MCHC)</td>
<td>33 ± 1.5 gm/dL</td>
</tr>
<tr>
<td>Reticulocyte Count</td>
<td>0.5 – 2.5 % [(2-2.5) x 10^7/L]</td>
</tr>
</tbody>
</table>

The earliest blood formation occurs in the primitive yolk sac but by the 5th-6th gestational week it becomes established in the liver which serves as the major site until about the 6th fetal month. During the middle trimester, there is some formation of blood in the spleen as well as the liver. Hemopoiesis starts in the bone marrow by about the fourth to sixth fetal month and this becomes the major organ of blood production from the sixth month onwards. The changing sites of hemopoiesis result from the migration of stem cells through the blood stream. Shortly after birth, all vestiges of hemopoiesis are lost from the spleen and liver although there may be some reversion to red cell production in these sites if there is anemia early in infancy.

Table 3: Biological reference interval (normal value) for the white cell series in adults

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Leucocyte Count</td>
<td>(7 + 3) x 10^9/L</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>40-80% [(1.8-7.8) x 10^9/L]</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>20-40% [(0.8-4.8) x 10^9/L]</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2-10% [(0.08-0.8) x 10^9/L]</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1-6% [(0.02-0.5) x 10^9/L]</td>
</tr>
<tr>
<td>Basophils</td>
<td>0-2% [&lt;0.2 x 10^9/L]</td>
</tr>
<tr>
<td>Platelets</td>
<td>(150-400) x 10^9/L</td>
</tr>
</tbody>
</table>

During the first few years of life, cellular bone marrow extends throughout the long bones, ribs, sternum, skull and vertebrae. After the age of four to five years, however, cellular marrow starts to be replaced by fat, first in the diaphyses of the peripheral long bones. This process extends centrifugally until by the age of 18 years, active marrow is found only in the vertebrae, ribs, sternum, skull and proximal epiphyses of the long bones. If there is an increased rate of erythropoiesis (production of red blood cells) in response to anemia, this fatty marrow may be replaced by active hemopoietic tissue.
The production of each mature cell is achieved by sequential mitosis from progenitor cells, as mentioned above. At subsequent steps of maturation, the cells within a group show common characteristics, such as reduction in cell size, loss of nuclei and gaining of specialized functions (Fig. 2). Whereas, most of these series continue to be produced by the bone marrow, after birth the lymphocytes are produced in lymph nodes, spleen and thymus.

**Regulation of hemopoiesis**

As mentioned earlier, the number of circulating cells, white cells and platelets remains within a relatively constant range (Tables 1 and 2). Certain physiological stimuli, such as exercise and emotional stress, may cause an immediate increase in the red cell and white cell (leucocyte) counts in the blood. It, hence, follows that a well-regulated mechanism must exist which controls the rate of production, release and egress of mature end cells from the hemopoietic organs (Fig. 8). This regulation may be attributed to local environmental factors and humoral factors, and is mediated by the co-operative interaction between intracellular repressor genes, extracellular local factors, humoral factors and chalones which regulate mitotic activity. The resultant mechanism that comes into play may be any of the following, or a combination thereof:

- more stem cells may differentiate into mature cells
- resting cells may enter the generative cycle
- the generative cycle may be quickened by shortening of the post-mitotic period
- mobilization of cells from sequestered pools.

**a) Regulation of red cell count** is by a feedback mechanism which may be a functional feedback (generated from the tissues) or an end-product feedback (generated from the RBC’s themselves).

**Functional feedback**: An increase in oxygen tension in tissues causes a decrease in the red cell count whereas a decrease in the oxygen tension causes an increase in the red cell count. Tissue hypoxia stimulates the cells of the juxtaglomerular (JG) apparatus in the kidney to produce erythropoietin. This is a non-dialyzable, thermostable $\alpha$-globulin first described in 1906 by Carnot and Beflandre. It has a molecular weight of 68000 daltons, a specific activity of 10,000 units per milligram of protein, and a half-life of 6 hours. It is now believed that the precursor of erythropoietin is produced in the bone marrow and liver, whence it is released into the circulation. Under conditions of hypoxia, the JG apparatus releases a renal erythropoietin factor which activates the erythropoietin precursor to form the functional erythropoietin molecule. The mechanism of action of erythropoietin is multiple:

- Activates mitosis in committed stem cells
- Promotes speed of maturation of activated red cell precursors
- Promotes early release of reticulocytes into the circulation at high levels (of erythropoietin).

Testosterone has also been seen to increase erythropoietin levels, thus causing an increase in erythropoiesis.

**End-product feedback**: Products released through the destruction of red cells are believed to influence the rate of red cell production. In polycythemia (increased red cells in circulation), a negative feedback is exercised, probably by the production of an inhibitor of erythropoietin.
Fig 8: Normal hemopoiesis
(Modified from Wintrobe’s Hematology 11th ed.)
b) Regulation of white cells is modulated via an end-product feedback as in the case of RBC’s and also by means of a colony stimulating factor (CSF) and a leucocyte inducing factor. The CSF is a glycoprotein present in the human serum, urine and bone marrow. It acts on precursors of granulocytes and monocytes, stimulating their proliferation. This CSF was first described by Bradley and Metcalf in 1996.

The leucocyte promoting or inducing factor is probably a releasing factor, which causes an acute rise in blood granulocyte levels by the release of preformed cells into the blood. It is a non-dialyzable, heat-labile globulin.

Whatever the mechanism, the ultimate control of hemopoiesis is affected through alteration of the microenvironment of the specialized regions that exist within the hemopoietic organs, and it is in these regions that the actual process of cell proliferation takes place.

In addition to these regulatory mechanisms, Bullough described certain factors known as “chalones”. These are produced by the tissues for the purpose of inhibiting mitosis of cells and are tissue-specific.

All mature blood cells are incapable of further divisions, except in the case of lymphocytes which divide readily on specific stimuli.

Constituents of normal blood

As already mentioned, blood consists of several different types of cells suspended in plasma (Fig. 5). These formed elements of blood, or blood cells, are of three types:

- red blood corpuscles, also known as erythrocytes,
- white blood corpuscles, also known as myeloid or lymphoid cells, and
- platelets, also known as thrombocytes or megakaryocytes.

Their classification and morphological analysis was made possible by the studies of Ehrlich who, in 1877, described the use of aniline dyes for staining dried blood films.

White blood cells

The WBCs constitute the blood's mobile security system. Some WBCs are endowed with the curious ability to wiggle out of the bloodstream and back in again. The WBCs can move like an amoeba, slipping through thin walls of capillaries and wandering among cells and tissues. They converge together in great numbers wherever invading bacteria, viruses, fungi, or parasites gain entry into the body, destroying them by swallowing them or by synthesizing antibodies, which are complex proteins that react with and destroy these foreign substances. Whenever white cells mobilize for action, the body compensates by manufacturing more.

Five different types of leucocytes are found in the peripheral blood, although it is convenient to consider them in two main groups:

- the phagocytes which have the capacity to engulf micro-organisms and other foreign material, and
- the lymphocytes or immunocytes which are concerned with the immune responses.
The phagocytes can be further subdivided into polymorphonuclear leucocytes, which are cells that have lost the capacity to replicate and whose nucleus has condensed into two or more separate lobes, and the monocytes, which are precursors of the much longer-lived tissue macrophages.

A characteristic feature of the phagocytes is the presence of a large number of cytoplasmic granules which are much more striking in the polymorphonuclear series, allowing the identification of these major types – the neutrophils, eosinophil and basophil – according to the staining characteristics of the cytoplasmic granules. In fact, the term granulocyte is often used to refer to these cells, or more specifically to the neutrophils, which are by far the most numerous as indicated in Table 1/2.

Unlike erythrocytes and platelets, the leucocytes are merely passengers in the blood stream and carry out their major functions within the tissues.

In the blood, phagocytes spend only a few hours in transit from the marrow to the tissues. Once there, the neutrophils survive only a few days, whilst the monocytes proliferate and differentiate into macrophages with a lifespan of months or years. Lymphocytes, on the
other hand, are generally much longer-lived and re-circulate many times between the lymphatic system and the bloodstream.

The neutrophil is akin to the infantry in a war – the first line of defense against infection. The principal function of the neutrophil is to ingest and kill bacteria by means of a process called phagocytosis. This involves contact between organism and neutrophil followed by membrane changes, which engulf the bacterium together with some of its surrounding milieu, containing it within a plasma membrane-lined vesicle or phagosome.

Phagocytosis is greatly enhanced when the organism is coated by immunoglobulin or complement. Then the process is called opronization, where the Ig or complement on the organism. The process of phagocytosis is followed by death and digestion of the ingested organism, which is achieved by cytoplasmic granules fusing with the phagosome and discharging their contents wherein whereby the bacterium is exposed to a wide variety of enzymes and cationic proteins. All bacteria do not succumb to these. The most potent bacteriological mechanisms are those that involve the generation of hydrogen peroxide or any of the reactive oxygen species. This process is followed by the degeneration of the neutrophil itself, releasing its granular contents amongst which is a potent pyrogen.

Accumulation of the degenerated neutrophils forms pus and the released pyrogen leads to increased body temperature, i.e. pyrexia or fever.

Eosinophil is the white blood cell that is responsible for combating infections by parasites. It has a bilobate nucleus and coarse granules which stain red using the hamanowsky method. Thes granules contain:
- histamine
- eosinophil peroxidase
- ribonuclease (Rnase)
- deoxyribonuclease (DRNase)
- lipase
- plasminogen
- major basic protein (MBP) which is toxic to both parasite and host tissues.

**Functions of eosinophils**
- They play a role in fighting viral infection by virtue of Rnase.
- Are involved in allergic response by virtue of histamine; they are also main effector cells in asthma pathogenesis.
- They facilitate fibrin removal by the action of plasminogen (which is a fibrinolytic agent).
- They fight helminth conolization.

Eosinophila is the presence of more than 500 cells/µL and happen in a variety of conditions like –
- viral infections
- allergic states
- helminthic infestations.

Basophils are the least numeroces of the leucocytes, very similar in appearance to neutrophils except for the various strikingly basophiliegranules. These granules contain mainly –
When basophils are triggered, they release two kinds of mediators:
1. Preformed granule associated mediators such as histamine, serotoxin, bradykinin, heparin cytokines (e.g. Interleukin – 4 or IL-4).
2. Newly generated mediators (prostaglandin and leukotrienes) made from arachidonic acid in surrounding tissue.

They play a crucial role in immediate hypersensitivity reactions. They have membrane receptor for IgE and the interaction of basophil bound IgE with specific antigens causes degranulation of the basophil and release of the granule-contents into the surrounding tissue. The clinical effects are dramatic and immediate – urticaria, bronchial asthma, rhinitis or in severe instances anaphylactic shock.

The peripheral blood lymphocytes plays a fundamental role in the immune response, its many subclasses interacting in a highly orchestrated fashion in responses to a variety of immunological challenges. The 2 major classes of lymphocytes are the T lymphocytes and the B lymphocytes, their named as the former are thymus dependent and are principally concerned with cell-mediated immune process. The B lymphocytes are involved in humoral immunity and are the precursors of antibody-producing plasma cells.

Monocytes, like the neutrophils, spend only a few hours in the blood in transit from the bone marrow to the tissues. They are the precursors of tissue macrophages, which, though widely distributed, have a pre-dilation for the lymph nodes, spleen, liver and marrow. Some even yet fixed to the endothelial lining of these organs and constitute the reticulo-endothelial system. The free macrophages respond readily to chemotactic stimuli, arriving at the inflammatory sites rather later than the neutrophils. They are able to process ingested antigen and stimulate the trans-formation of virgin lymphocytes into specifically sensitized cells. The also respond to various humoral mediators (lymphokines) liberated by T lymphocytes.

**Platelets**

The main function of the platelets is to maintain haemostasis. Elements of the bilamellar plasma membrane interact with coagulant proteins and allow then a platform for the successive reaction for fibrin production. Platelet adhesion occurs at the site of de-endothelialization, so as to maintain integrity of vascular compartment. Damage to the vessel wall, in addition to causing platelet adhesion trigger, a further series of reactions during which the platelet extends active agents to recruit other platelets into the haemostatic plug. Hence, platelets perform a three-fold function of haemostasis, namely:

- Adhesion
- Aggregation
- Interaction with the coagulation mechanism.

Inactive platelets circulate as bioconvex discs. They are about one-tenth the volume of RBC and about twenty times less numerous.
**Lymphocytes**

These are a type of white blood cells involved in the body’s immune system and play an integral role in the body’s defenses. They have a large dark-staining dense nucleus.

They are of two types, the T cells and the B cells, which mature in the thymus and the bone marrow, respectively. In the presence of antigen the B cells can become much more metabolically active and differentiate into plasma cells, which secrete large quantities of antibodies. Microscopically, it is impossible to distinguish between T cells and B cells, which requires flow cytometry.

Usually, lymphocytes increase in number in viral infections, but the human immunodeficiency virus (HIV) actually hijacks and destroys T cells specifically (CD4 lymphocytes). Without this defense, the body is susceptible to opportunistic diseases that otherwise would not kill healthy people.

**Biomedical aspects**

1. Double the usual number may appear in the blood within hours of an infective agent harboring in the body. This rising white cell count serves as an early tip-off that a dangerous infection has entered the body. The extent of the increase is, often, an indicator of the extent of morbidity of the infection and becomes a guideline for the clinician in treatment of the patient.

2. Sometimes the balance of mobilisation and manufacture turns awry, there is unprecedented production of the white blood series and leukaemia results. An increase in the WBC count of a great magnitude can warn of a this stem cell malfunction or overactivity.

**Red blood cells**

The red blood cells are biconcave circular cells (Fig. 10) containing the hemoglobin which gives the blood its red colour. They were first described in 1674 by the Dutch scientist Leeuwenhoek. Interestingly, this was the first step towards the discovery of blood. Leeuwenhoek observed the red colour in the web of a frog’s leg. When he pricked it, a red fluid oozed out and the web lost its colour. He, then, looked at this fluid through the lens that he had just made (he used to grind glass himself in the attempt to make lenses with a higher magnification which could be used in microscopes and helped his colleagues like Louis Pasteur) and discovered the biconcave cell, which is now known as a Red Blood Cell.

![Fig. 10: Red blood cell – size and shape](image)
It has two main functions, i.e. to maintain itself in circulation for 120 days and to keep its hemoglobin in a state suitable for oxygen transportation during that time. In describing the functions of the red cell, its three components need to be considered separately, namely the membrane, hemoglobin and the metabolic pathways. However, each of these is dependent on one another and can interact to modulate oxygen transport and maintain oxygen delivery to the tissues.

**Membrane** The red cell membrane, like all cell membranes, is a bipolar lipid layer containing enzymes. This configuration is called a ‘membrane-associated cytoskeleton’. The proteins in the membrane provide protection from various types of stresses, e.g. muscle contraction. It differs from other membranes in that it contains not only structural proteins but contractile proteins as well (Fig. 11).

![Fig. 11: The red blood cell membrane](image)

Spectrin, a contractile protein of the internal surface of the membrane, is responsible for the biconcave disc shape of the red cell, and abnormalities of spectrin may account for some hereditary conditions associated with shape changes of the red cell, e.g. hereditary spherocytosis, which lead to hemolytic anemia.

This contractile protein, spectrin, is a tetramer (Fig. 12) which comprises triple helicle repeats (Fig. 13) thus giving it its contractile property and this enables considerable distortion of the 7.5 µm-diameter cell so that it traverses the 3.4 µm-diameter splenic vessels, which are the smallest.

Interestingly, there are other sites, too, where spectrin exists and one of them is the heart muscle where it probably integrates the calcium ion-handling system that controls heart beating with the contractile apparatus that drives beating.
In addition to the above-mentioned properties and functions, the red cell membrane has yet another significant property – that of carrying the blood group antigens. Landsteiner was the first scientist to demonstrate their existence, for which he received the Nobel Prize in 1930.

These antigens are glycolipids or glycoproteins of molecular weight >40,000 daltons and are coded by specific genes. The type and number of antigens vary widely (Table 4) and hence, blood group systems have evolved which are genetically discrete, e.g. the ABO system, the Rhesus (Rh) system, the MNS system, the P system, the Lutheran system, etc. In fact, there are over 25 genetically discrete blood group systems, which have been numerically identified (in order of their discovery) by the working party on terminology for RBC antigens – a party sanctioned by the International Society of Blood Transfusion (ISBT). Thus, the ABO system has been given the number ‘001’, the MNS system ‘002’, the P system ‘003’, the Rh system ‘004’, the Lutheran system ‘005’ and so on. Of these, the systems of greatest clinical importance and significance are the ABO and the Rh systems.

In the ABO system, people are classified as the ‘A’ group, the ‘B’ group, the ‘AB’ group and the ‘O’ group (Table 5), depending on the presence or absence of the A, B and H antigens on the RBC and the corresponding antibodies in the serum. On basis of the presence or absence of the Rh antigens, people are classified as either Rh positive or Rh negative (Table 5). As already mentioned, these antigens are coded by specific genes and their inheritance follows the Mendelian Law, so that every individual is either homozygous or heterozygous for a particular blood group.
Table 4: Red blood cell antigens

<table>
<thead>
<tr>
<th></th>
<th>ABO system</th>
<th>Rh system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites on RBC</td>
<td>1,000,000 (10⁶)</td>
<td>10,000-33,000</td>
</tr>
<tr>
<td>Sites on RBC</td>
<td>A,B,H (H is a precursor of A and B)</td>
<td>D,C,E,d,c,e</td>
</tr>
<tr>
<td>Genes carrying their codes</td>
<td>3 genes on chromosome 9q</td>
<td>2 genes on chromosome 1p</td>
</tr>
</tbody>
</table>

Table 5: ABO and RH blood groups

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Discovered by/in (scientist / year)</th>
<th>Antigen on RBC</th>
<th>Antibody in plasma/serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Landsteiner/1900</td>
<td>A</td>
<td>Anti-A</td>
</tr>
<tr>
<td>B</td>
<td>Landsteiner/1900</td>
<td>B</td>
<td>Anti-B</td>
</tr>
<tr>
<td>AB</td>
<td>Von Decastillo/1902</td>
<td>A, B</td>
<td>None</td>
</tr>
<tr>
<td>O</td>
<td>Von Decastillo/1902</td>
<td>None</td>
<td>Anti-A, Anti-B</td>
</tr>
<tr>
<td>Rh positive</td>
<td>Levine &amp; Stetson/1939</td>
<td>D (mostly)</td>
<td>None</td>
</tr>
<tr>
<td>Rh negative</td>
<td>1939</td>
<td>None</td>
<td>Anti-D, but only after antigenic stimulus</td>
</tr>
</tbody>
</table>

As opposed to these major blood group systems, there are two minor systems, which warrant mention here as they are of importance in the Indian scenario; these are the Bombay and the Indian blood groups (Table 6).

Table 6: Special blood groups

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Reported in</th>
<th>Prevalence in India</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombay</td>
<td>1952</td>
<td>1:7600</td>
<td>Absence of A, B, H antigens on RBC; Presence of all corresponding antibodies in the serum.</td>
</tr>
<tr>
<td>Indian</td>
<td>1973</td>
<td>1:25</td>
<td>Antibodies present cause reduced RBC survival.</td>
</tr>
</tbody>
</table>

In this section, it becomes imperative to talk of the blood group antibodies as well, which circulate in the plasma.

Antibodies are immunoglobulins, which comprise about 20% of the total plasma proteins. Immunoglobulins (Igs) are proteins, which function as antibodies.
They are Y-shaped molecules and are comprised of two identical polypeptide chains of molecular weight 23000 (designated light chains) and another two polypeptide chains of molecular weight 55000 (designated heavy chain), each light chain being linked to a heavy chain by a disulphide bond. The C-terminal halves of the light chains categorise these into two distinct types – kappa (κ) and lambda (λ). In a usual mixture of Igs, about 70% of the light chains are κ and 30% are type λ. The N terminal is highly variable and its composition is unique to each individual antibody. Similarly the heavy chains, too, have a highly variable N terminal and a relatively constant C terminal, which allows a broad classification into five antigenically distinguishable forms: gamma (γ), alpha (α), mu (µ), delta (δ), and epsilon (ε) also known as IgG, IgA, IgM, IgD, and IgE, respectively. The former three are present in much higher concentrations than IgD and IgE.

These antibodies are produced in response to some antigenic stimulus. The factors influencing their production are:
- antigen size
- complexity of antigen molecule
- dose of antigen
- host HLA genotype

Blood group antigens are either carbohydrate-based, in which case they elicit a T-cell independent IgM response, or they may be protein based, in which case they elicit a T-cell dependant primary IgM response followed by a secondary IgG response.

The blood group antibodies are of two types – natural and immune. The introduction of red cell antigen into the circulation of an individual lacking that antigen may stimulate the production of a corresponding antibody. This may occur as a result of blood transfusion therapy, or fetomaternal blood group incompatibility in pregnancy. These are called the incomplete or acquired antibodies (IgG). The anti-rhesus antibody is an example of this variety. These react best at 37°C and require antihuman globulin for detection.

On the other hand, certain antibodies occur without known antigenic stimulus. These are known as complete or natural antibodies (IgM, IgA). Theoretically, these antibodies are...
produced in response to substances in the environment which are genetically identical with or similar to red cell antigens. The antibodies to the ABO, the MN and the P blood group antigens are examples of this type of antibodies. The common occurrence of these naturally occurring antibodies suggests that their antigens are abundant in nature in the form of animals, bacteria, pollen, etc. Unlike the acquired antibodies, these natural blood group antibodies are cold agglutinin of the IgM subtype, which react better at room temperature or below, and activate complement components.

The importance of the blood group antibodies lies in their ability to cause transfusion reactions. Depending on the type of blood group antibodies one has, one can receive from or donate blood to individuals of only particular blood groups.

<table>
<thead>
<tr>
<th>RECIPIENT</th>
<th>DONORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A, O</td>
</tr>
<tr>
<td>B</td>
<td>B, O</td>
</tr>
<tr>
<td>AB</td>
<td>AB, O</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

The importance of blood group antibodies:
1. Blood Transfusions
   a. Cross matching can have major or minor incompatibilities.
   b. Complications can be hemolytic reactions, febrile reactions, hyperkaemia, hypocalcemia and transmission of disease.
2. Hemolytic disease of the newborn which may manifest as erythroblastosis fetalis, which is a result of Rh incompatibility between mother and foetus, or as fetal ABO hemolytic disease due to ABO incompatibility between the mother and the foetus.
3. Haemoglobin During the production of red blood cells from the unipotent stem cell or erythroblast, it passes through various stages of maturation, namely pronormoblast, basophilic normoblast, olychommatophilic normoblast, orthochromat normoblast, reticulocyte and, finally, erythrocyte.

During these phases, the following changes occur:
1. The size reduces from 20-25 µm to 7-9 µm
2. Reduction in size of nucleus occurs till it is totally absent in the reticulocyte.
3. Hemoglobinization begins in the polychromatophilic normoblast and slowly increases till the final stage of maturation.
4. Biochemically, the cells, during maturation, lose their ability to synthesize nucleic acids, nucleotides and proteins, as well as the succinate dehydrogenase and cytochromes involved in the tricarboxylic acid cycle and electron transport, respectively.

As already mentioned, the main function of the red blood cell is to maintain the hemoglobin in a state conducive to transport of gases to and from various tissues. The energy required for these is derived from anaerobic glycosis and the hexose and pentose pathways, which are the only metabolic processes occurring in the mature red blood cell.

Max F. Perutz (1914-2002), an Austrian-born British Biochemist won the Nobel Prize for elucidating the three-dimensional (3-D) structure of proteins, especially for his work on the hemoglobin molecule (Fig. 15).
The hemoglobin is an iron-protein, which transports oxygen (O$_2$), carbon dioxide (CO$_2$) and nitric oxide (NO) to and from the cells and the lungs. When it takes up O$_2$ from the lungs it is called oxyhemoglobin (which imparts the bright red colour) and when it takes up CO$_2$ or carbon monoxide (CO) from the tissues it becomes carboxy-hemoglobin, which is of a dark cherry-maroon colour and accounts for the cyanosed (bluish) appearance of the skin under conditions of hypoxia. In addition to this prime function, in 1996 hemoglobin was found to transport NO as well. Nitric oxide is a vasodilator and thus hemoglobin helps in regulating blood pressure by controlling the amount of NO the vessels are exposed to.

Fig. 15: 3-D Structure of hemoglobin

Hemoglobin carries more than 20 times its volume of oxygen. Some chemicals as well as CO combine so firmly with hemoglobin that it can no longer combine with oxygen, and asphyxiaition results. The molecule is a spherical globin, which carries iron in the ferrous form within the globe. Its oxygen-carrying capacity is dependent on its structure and, hence, a description of the molecule is imperative.

Hemoglobin is a tetramer of 2 alpha (α) and 2 beta (β) globin chains consisting of 141 and 146 amino acids, respectively (Fig.15). Each globin chain contains a haem molecule with an iron atom, which can reversibly bind to oxygen. It is the subunit interactions that are at the heart of its ability and capacity to carry oxygen (O$_2$), carbon dioxide (CO$_2$), nitric oxide (NO) and hydrogen ions (H$^+$) in a physiologically responsive way.

The affinity of the molecule is, in addition, affected by pH, temperature and 2,3-biphosphoglycerate (BPG) concentration. These factors facilitate O$_2$ uptake in the lungs and its release into the tissues. Changes in the polypeptide subunits of the globins can also affect the affinity of the hemoglobin molecule for oxygen. For e.g. foetal hemoglobin (HbF) has two gamma (γ) chains instead of the two β chains (Table 7).

This substitution increases its affinity for oxygen due to a less strong binding to 2,3-BPG, so that O$_2$ is preferentially transferred to HbF at the maternoplacental interface. Thus, oxygen delivery to the foetus is priority-based. This is a physiological variation, but, at times, there are pathological changes in the polypeptide units, which are the bases of disease
states such as sickle cell disease and the thalassemias. The normal, embryonic and abnormal globin chains that may be present in various types of the hemoglobin molecules are, respectively, enumerated in Tables 7, 8 and 9.

Table 7: Normal hemoglobin

<table>
<thead>
<tr>
<th>Type</th>
<th>Globin chain</th>
<th>Percentage in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2 alpha, 2 beta (α2, β2)</td>
<td>96%</td>
</tr>
<tr>
<td>A2</td>
<td>2 alpha, 2 delta (α2, δ2)</td>
<td>2.5-3%</td>
</tr>
<tr>
<td>F</td>
<td>2 alpha, 2 gamma (α2, γ2)</td>
<td>1-1.5%</td>
</tr>
</tbody>
</table>

Table 8: Embryonic Hb’s (Normal)

<table>
<thead>
<tr>
<th>Type</th>
<th>Globin chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Portland</td>
<td>2 zeta, 2 gamma (ζ2, γ2)</td>
</tr>
<tr>
<td>Hb Gower – 1</td>
<td>2 zeta, 2 epsilon (ζ2, ε2)</td>
</tr>
<tr>
<td>Hb Gower – 2</td>
<td>2 alpha, 2 epsilon (α2, ε2)</td>
</tr>
</tbody>
</table>

Table 9: Some common abnormal hemoglobins

<table>
<thead>
<tr>
<th>Type</th>
<th>Functional anomaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbS</td>
<td>Abnormal sickle Hb has a tendency to undergo polymerization, leading to distortion of red cells, altered exterior and, hence, altered oxygen carrying capacity and sickle cell formation.</td>
</tr>
<tr>
<td>HbM</td>
<td>Altered active site and hence direct alteration of O2 binding.</td>
</tr>
<tr>
<td>HbH</td>
<td>Altered tertiary structure (i.e. polypeptide folding) Altered quaternary structure due to mutations at the subunit interfaces. Thus, there is indirect alteration of oxygen binding.</td>
</tr>
<tr>
<td>HPFH</td>
<td>When the HbF persists after birth in higher concentration than normal (hereditary persistence of fetal hemoglobin), oxygen delivery to the tissues is affected as the molecule has a high affinity for O2.</td>
</tr>
<tr>
<td>Thalassemias</td>
<td>Altered synthesis of either type of globin chain resulting in low Hb production and variable susceptibility to hypoxia.</td>
</tr>
</tbody>
</table>

Since the primary, secondary and tertiary structure of the molecule would have been dealt with in other sections of this compilation, here only the quaternary structure will be considered.

The 64,500 dalton 55 Å spherical hemoglobin molecule holds its haem groups in crevices near the exterior of the molecule (one in each subunit), which position enables them to take up and release O2 and CO2. This gives the molecule a tetrahedral array - Nature’s “Fundamental Design” for an oxygen carrier.
The genes that code for the various polypeptides of hemoglobin are present on chromosome 16 (for α and ζ globin chains) and on chromosome 11 (for β, γ and δ globin chains). Any point mutation or alteration in the polypeptide coding in the genes leads to an altered type or amount of α or β chain, which affects, directly or indirectly, the oxygen carrying capacity of the molecule.

Here it is pertinent to mention that the genes coding for myoglobin and hemoglobin (both oxygen carriers of varying capacity) are variations on a fundamental theme. Hence, they are an example of gene duplication and diversification.

Why is this termed the “Fundamental Design” of nature for oxygen carriers? And how does the monomeric myoglobin oxygen carrier differ from the tetrameric hemoglobin oxygen-carrier?

Myoglobin, a protein of the red muscle, stores oxygen as a reserve against oxygen deprivation; hemoglobin, on the other hand, transports O₂ to the tissues and returns CO₂ and protons to the lungs. Cyanide and carbon monoxide kill because they disrupt the physiologic function of the haem protein’s cytochrome oxidase and hemoglobin respectively.

The secondary and tertiary structure of hemoglobin resembles that of myoglobin, but the tetrameric structure of hemoglobin permits co-operative interactions that are central to its function. Hemoglobin described as an allosteric protein because of the intricate interaction of the different subunits.

![Fig. 16: Oxygen dissociation of myoglobin and hemoglobin](image)

It is evident here that the myoglobin molecule gets saturated at a much lower concentration of oxygen than hemoglobin does due to its higher affinity for oxygen. Hence its oxygen dissociation curve is hyperbolic (resulting in a less oxygen delivery) whereas that of hemoglobin is sigmoidal and its oxygen delivery is better.
This makes it differ from simpler monomeric protein myoglobin in 3 ways:

1. The binding of O\(_2\) to hemoglobin enhances the binding of additional O\(_2\) --- “cooperative binding”.
2. The affinity of hemoglobin for O\(_2\) depends upon pH (i.e. hydrogen ion concentration) as well as CO\(_2\), presence of either promoting a release of O\(_2\) form its binding to hemoglobin. Reciprocally, O\(_2\) promotes the release of bound hydrogen ions (H\(^+\)) and CO\(_2\) (Fig. 17).

Thus, the ability of hemoglobin to release oxygen is affected (as mentioned above) by the pH, CO\(_2\) and by the difference in the oxygen-rich environment of lungs and the oxygen-poor environment of the tissues. The pH in the tissues is considerably lower (more acidic) than in the lungs. Protons are generated from the reaction between carbon dioxide and water to form bicarbonate.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+
\]

This increased acidity serves a twofold purpose. First, protons lower the affinity of hemoglobin for oxygen, allowing easier release into the tissues. As all four oxygen ions are released, hemoglobin, binds to two protons. This helps to maintain equilibrium towards the right side of the equation. This is known as Bohr Effect (Fig. 17) and is vital in the removal of carbon dioxide as waste, because CO\(_2\) is insoluble in the blood stream. The bicarbonate ion is much more soluble, and can thereby, be transported to the lungs after being bound to hemoglobin. If hemoglobin could not absorb the excess proton, the equilibrium would shift to the left, and carbon dioxide would not be removed.
The loss of anionic bicarbonate from the RBC is compensated (maintaining electrical neutrality) by an influx of chloride ions (anions) – the so called “Chloride Shift”. In the lungs, the Bohr effect works in the reverse direction. In the presence of the high oxygen concentration in the lungs, the proton affinity decreases. As protons are shed, the reaction is driven to the left, and CO₂ forms as an insoluble gas to be expelled from the lungs. The proton-poor hemoglobin now has a greater affinity for O₂ and the cycle continues.

3. The O₂ affinity of hemoglobin is further regulated by organic phosphates like 2,3-biphosphoglycerate(BPG), an increased concentration of which promotes oxygen delivery to tissues.

In contrast, the binding of O₂ to myoglobin is independent of these factors. Hence the difference in the oxygen dissociation curves of the two proteins (Fig. 18).

![Fig. 18: Oxygen dissociation curve and its determinants](image)

Not only is the binding of oxygen cooperative, but so is the unloading of O₂ in tissues where the unloading of O₂ from one haem facilitates the unloading of O₂ from another haem in the same tetramer. This enables hemoglobin to deliver 1.83 times as much oxygen (under typical physiological conditions) as it would if the sites were independent.

**Metabolic pathways**

By far the most important metabolite in the RBC is 2,3-BPG which also exerts a modulatory effect on the oxygen dissociation from hemoglobin. A low p O₂ in the tissues promotes the formation of 2,3-BPG from the glycolytic intermediate 1,3-BPG. The accumulation of 2,3-BPG in the RBC reduces the affinity of hemoglobin for O₂ and therefore causes oxygen to dissociate from the hemoglobin molecule and be delivered to the tissue for respiration. At
the same time, the CO₂ and proton released during tissue respiration are taken up cooperatively by the hemoglobin molecule and delivered to the lungs. In the absence of BPG, the O₂ dissociation curve of hemoglobin mimics that of myoglobin, indicating a high affinity of hemoglobin for O₂ and therefore an inability to deliver the oxygen.

**Biomedical aspects of RBC**

1. **Anemias** are a group of disorders characterized by a decreased delivery of O₂ to tissues (tissue hypoxia) which may be caused by deficiency states (most commonly of iron, folic acid or B₁₂) leading to delayed or impaired RBC production or hemoglobin synthesis. It may also be caused by defective RBC like sickle cells, whose O₂ carrying capacity is altered.

2. **Hemoglobin disorders**
   a) **Thalassemias** result from low to no production of one or both types of the globin chains. These are due to genetic defects and so far 750 different mutations have been identified.
   b) **Hemoglobinopathies** are characterized by single nucleotide substitutions on the globin chains, which alter the oxygen carrying capacity of the tetramer.

**Haemostasis**

When there is a breach in the channel system of circulation, one would expect blood to be lost continuously as it passes through that sector because of the higher intravascular pressure. Evolution has resulted in the development of an efficient, but complex series of hemodynamic, cellular and biochemical mechanism, which limit blood loss. What actually happens is that, initially, blood does start oozing out of the breached site, but soon (within a few seconds) a series of reactions is initiated in response to the contact with the breached site. These reactions are, in turn, regulated by feedback-modulated reactions. The result is a solidification of the otherwise fluid blood i.e. thrombosis. But, as soon as the breach is obliterated, the reactions stop as though a switch has been turned off. Only under extreme circumstances does the response outrun the challenge and thrombotic material extend inappropriately into the undamaged circulation.

Any imbalance in any of the series of reactions would either lead to uncontrolled bleeding at the site of breach or, as mentioned above, to inappropriate extension of the thrombotic material.

The haemostatic mechanism has four components, viz. the vascular component, the platelet, the coagulation sequence and the fibrinolytic system.

**The Vascular Component** is comprised of a few different mechanisms, which proceed simultaneously.

a) **Vasoconstriction** occurs immediately at the site of injury and this is dependent on the stretch receptors in the smooth muscle fibres. In fact, not only does the affected vessel constrict, but so do the other vessels in the surrounding area, limiting blood flow to the injured segment and, thereby, limiting its loss. Serotonin and thromboxane A₂ (TX A₂) released from platelets are probably the main effectors of this vessel wall response.
b) **Inhibitor systems:** The luminal surface of the endothelial cells lining the blood vessels are coated with glycosaminoglycans which are complex mucopolysaccharides with elaborate repetitive long-chain structure and they carry a negative charge, e.g. heparin. Heparin is a mucopolysaccharide with a molecular weight ranging from 6,000 to 40,000 Da. The polymeric chain is composed of repeating disaccharide units of D-glucosamine and uronic acid linked by 1→4 interglycosidic bonds. Few hydroxyl groups on each of these monosaccharide residues may be sulfated giving rise to a polymer that is highly negatively charged.

The key structural unit of heparin (Fig. 19) is its unique pentasaccharide sequence. This sequence consists of three D-glucosamine and two uronic acid residues.

![Fig. 19: Structure of heparin](image)

The central D-glucosamine residue contains a unique 3-O-sulfate moiety that is rare outside of this sequence. Four sulfate groups on the D-glucosamines are found to be critical for retaining high anticoagulant activity.

The endothelial cells also synthesize two activators of the fibrinolytic enzyme system, which give them an apparently, non-responsive character. Also, the platelets have little tendency to adhere to the endothelium in vivo. This is probably due to the production of enzymes with ADPase activity by the lining cells of the vessels. This limits the inter-platelet reactions, too.

An even more potent inhibitor of platelet reaction is the prostaglandin I$_2$, PGI$_2$, or prostacyclin, again synthesized in the endothelial cells, which has the additional action of preventing platelet surface adhesion.

c) **Response to Injury:** *Tissue thromboplastin* is the most important of the substances released from the vessel wall following injury. It is a lipoprotein that promotes haemostasis through combination with factor VII as shown in the cascade of reactions in (Figure XXI). This sequence of reactions is extremely rapid and this tissue factor is the major haemostatic capital.

**Biomedical aspects**

1. Heparin, containing the unique five-residue sequence forms a high-affinity complex with antithrombin. The formation of antithrombin - heparin complex greatly increases (about a thousand-fold) the rate of inhibition of two principle procoagulant proteases, factor Xa and thrombin. Accelerated inactivation of both the active forms of these proteases prevents the
subsequent conversion of fibrinogen to fibrin that is crucial for clot formation. This is the basis of its use in patient care.

2. At the time of crush injury, damage to the blood vessels is extensive. This leads to a massive release of tissue thromboplastin causing the development of disseminated intravascular coagulation (DIC), a hypercoagulable state also known as Consumptive Coagulopathy or Thrombohaemorrhagic Syndrome. This can result in death.

d) **Endothelial cells** also synthesize the *part of factor VIII molecule*, which is concerned with platelet surface reactions. Platelets have a specific surface receptor for the factor and its release from the endothelial cells at sites of damage contributes to the formation of the platelet haemostatic plug.

**The Platelet** These tiny biconvex cells are the first line of defence in haemostasis. They, actually, adhere to any foreign surface as well as to the constituents of the subendothelium, particularly collagen. Therefore, damage to the vascular endothelium results in a monolayer of platelets adhering to the exposed collagen – a calcium-dependant irreversible process mediated by Von Willebrand Factor (VWF). It forms links between the platelet glycoprotein receptor and the collagen fibrils. This is called **Primary Haemostasis** (Fig. 20). This happens by virtue of the property of **adhesion** of platelets. If this monolayer is sufficient to bridge the breach, haemostasis stops at adhesion. But, if the injury is more extensive, platelet adhesion and activation of coagulation occur concurrently. Release of stored amines and synthesized prostanoids following adhesion of single platelets causes other platelets in the immediate vicinity to become sticky and adhere to platelets that release these substances and to each other. This is the process of **platelet aggregation**. In this way, a hemostatic plug is built up at sites of injury. As the platelet microthrombus forms, it is enmeshed in strands of fibrin which firmly cement the thrombus and prevent its premature dissolution. Since, fibrin is the final product of coagulation, the two processes occur hand-in-hand.

![Fig. 20: Primary hemostasis](image)

**Breath in endothelial cell lining or basement membrane** Causes platelets to adhere to exposed collagen.

**On adhesion, platelets release ADP and TXA2** which are chemotactic for platelets in the vicinity which also adhere to the site of breach as well as the platelets, thus forming a primary hemostatic plug.

In addition to this, the platelets directly interact with the coagulation mechanism in two different ways.
• in the generation of factor Xa (activated factor X)
• as a catalytic in the generation of thrombin by the factor Xa complex.

Coagulation Sequence  As is evident from the above discussion, coagulation or secondary haemostasis (Fig. 21) is an amalgamation of the Intrinsic Pathway (also known as the Contact Activation Pathway), and the Extrinsic Pathway (also known as the Tissue Factor Pathway)

These pathways are a series of reactions, in which a zymogen of a serine protease and its glucoprotein cofactor are altered to become active components that then catalyze the next reaction in the cascade [Coagulation factors are generally indicated by Roman numericals, with a lower case ‘a’ appended to indicate an active form]

As is shown in the cascade, two pathways are initiated almost simultaneously – one at the vessel wall and the other in the tissue. In the intrinsic pathway, the exposed collagen in the vessel wall promotes the activation of factor XII in the presence of kallikrein and kininogen. Factor XIIa, in turn, promotes the activation of factor XI and factor XIa then activates factor IX.

At the same time, in presence of tissue factor, factor VII is activated. Now, the factors XIa and VIIa, along with the inactive as well as activated forms of factor XIII, converge in the cascade to together activate factor X. Again the cascade continues, with factor Xa promoting activation of factor V and factor Va, then aiding the conversion of prothrombin (factor II) to thrombin (factor IIa). The action of thrombin is dual; along with calcium, it helps activate factor III, and it also promotes the conversion of fibrinogen (factor I) to the monomer fibrin (factor Ia). These fibrin monomers group together (in the presence of factor XIIIa) to form fibrin multimers, which interlace with each other to form a cross-linked fibrin mesh called a ‘clot’.

Nomenclature of factors of coagulation

| Factor I (F I) | Fibrinogen |
| Factor II (F II) | Prothrombin |
| Factor ? | Tissue Factor (TF) |
| Factor ? | Calcium (Ca++) |
| Factor V (F V) | Proaccelerin / labile factor |
| Factor VI (F VI) | Unassigned (old name for F Va) |
| Factor VII (F VII) | Antihemophilic Factor (AHF) |
| Factor VIII (F VIII) | Hemophilic Factor |
| Factor IX (F IX) | Christmas Factor |
| Factor X (F X) | Stuart-Prower Factor |
| Factor XI (F XI) | Plasma Thromboplastin Antecedent (PTA) |
| Factor XII (F XII) | Hageman Factor |
| Factor XIII (F XIII) | Fibrin-Stabilising Factor |

The Intrinsic Pathway is initiated by the platelets when they come in contact with the exposed collagen at the site of injury. The series of reactions that occur have already been discussed above.

On the other hand, the Tissue Factor Pathway mainly generates thrombin. This constituent of the coagulation cascade is probably the most important in that it is the one factor formed
by feedback control. It has a large array of functioning, primarily the conversion of fibrinogen to fibrin, the building block of a hemostatic plug.

**Fig. 21: Hemostasis**

The coagulation pathway has some co-factors and inhibitors. The co-factors are:

- Calcium
- Phospholipid
- Vitamin K

The inhibitors are:
- **Protein C** degrades factors Va and VIIIa
- **Protein S** activates protein C
- **Antithrombin**, a serine protease inhibitor (Serpin), degrades thrombin and factors IXa, Xa, Xla and XIIa.
- **Heparin** promotes the affinity of antithrombin for these factors.
- **Tissue Factor Pathway Inhibitor** (TFPI) inhibits factor VIIa-related activation of factors IX and X.

[Antithrombin is a single-chain glycoprotein of 425 amino acids. Its inactivation of thrombin is irreversible and achieved by the formation of a stable complex in which both the proteins are inactivated. It is synthesized in the liver and its decreased level (or decreased activity) in plasma results in a hypercoagulable state manifesting as various forms of thromboembolic diseases.]

All these promotions and inhibitions occur side-by-side and are, thus, in a state of dynamic balance, which tightly controls the process of coagulation to suit the needs of the system in that part of the body at that point of time.

When blood is drawn from a blood vessel and allowed to stand, the process of hemostasis causes the formation of a solid clot and a supernatant fluid. It is evident that the supernatant fluid is plasma devoid of fibrinogen; this is called *serum*.

**Fibrinolytic system**

This system, as the name suggests, limits excessive fibrin formation through plasmin mediated fibrinolysis. Circulating plasminogen binds to fibrin and is converted to active plasmin by tissue plasminogen activator (tPA) secreted by the endothelial cells. The activation occurs most effectively only when plasminogen and its activator (tPA) bind to the fibrin in the clot, thus localizing its activity to these fibrin deposits. The specific action of tPA is inhibited/suppressed in the plasma by the presence of its inhibitor – plasminogen activator inhibitor (PAI) which is synthesized by endothelial cells as well as hepatocytes.

**Plasma**

Having dealt with the cellular elements of the blood, we now turn our attention to the plasma, the milieu in which these cellular elements are constantly bathed.

Constituents of plasma can be summarised as below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>90%</td>
</tr>
<tr>
<td>Protein</td>
<td>8%</td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>0.9%</td>
</tr>
<tr>
<td>Organic substances</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

Theae plasma proteins can further be subdivided into 3 types:

- Albumin 60%
- Globulin 36%
- Fibrinogen 4%

The major inorganic ions along with their concentration in the plasma are listed below:

- **Sodium (Na)** 135 – 148 mmol/L
- **Potassium (K)** 3.5 – 5.5 mmol/L
- **Calcium (Ca)** 2.1 – 2.7 mmol/L [8.2 – 10.4 mg/dL]
- **Chloride (Cl)** 98 – 108 mmol/L
- **Bicarbonate (HCO₃)** 23 – 31 mmol/L
- **Phosphate (PO₄)** 0.7 – 1.4 mmol/L [2.5 – 4.6 mg/dL]
The electrolytes constituting the plasma are of prime importance in the maintenance of homeostasis – the mechanism by which the intracellular contents are maintained in equilibrium with the extracellular contents.

Amongst the nutrients carried in the plasma, the major ones are:

- **Glucose** (primary source of energy for cell metabolism) 70 – 160 mg/dL
- **Amino acids** (building blocks for protein synthesis) 120 – 190 mg/dL
- **Cholesterol** 35 – 160 mg/dL
- **Triglycerides**
- **Vitamins**

At the same time, the waste products that are transported are:

- **Urea** (nitrogenous waste from breakdown of proteins) 25 – 40 mg/dL
- **Uric acid** 2.0 – 5.7 mg/dL in females, 2.4 – 7.2 mg/dL in males
- **Creatinine** 0.6 – 1.3 mg/dL

The effector substances transported in the blood stream are mostly hormones (e.g. cortisol, thyroxine, ADH, insulin, etc.) and all drugs, whatever the mode of administration.

We know that, dissolved in the aqueous plasma, the blood carries all the necessary nutrients and ‘messengers’ (hormones) to all the cells of the body, but having done so, how do these substances leave the channels of circulation and reach their destination? This feat is achieved through various controlling factors, viz.

- the size of the molecule to be transported across the membrane
- the ionic gradient across the capillary membrane (this is different for every type of ion)
- the osmotic pressure on either side of the membrane (i.e. the osmotic gradient) which is given, largely, by the protein molecules
- the hydrostatic pressure/ gradient, which is a function of the aqueous medium.

The osmotic and hydrostatic pressures are, of course, reciprocal, i.e. the greater the hydrostatic pressure, the lower is the osmotic pressure and vice versa. And then there are the ions, which flow freely across some membranes and not at all across others. Nature has devised a complex system of exchange at the cellular level, which is self-regulatory. We have already reviewed one example – the delivery of oxygen to the cells.

Though most of the substances in the plasma are nutrients that are only passing through these channels to their ultimate destination, there are many substances which are integral constituents of the plasma, e.g. plasma proteins.

**Plasma proteins** are a highly complex group of molecules whose functions are as vast as they are varied.

1. **Nutrition**: The amino acid pool derived from their breakdown are used not only for synthesis of new proteins but also for synthesis of alternate sources of energy for metabolism in absence of direct sources of energy.
2. **Colloid osmotic pressure of plasma proteins** and polypeptides maintains the volume of the intravascular component – albumin being the major contributor.
3. Transport: While albumin transports a wide range of substances, other proteins have specific transport functions. Hormones, fat-soluble vitamins, metals, drugs – all important substances in the body – are transported within the body by proteins.

4. Many of the coagulation factors are proteins.

5. Protection is mediated by immunoglobulins which are proteins.

6. Buffering of plasma pH is done by protein.

7. Enzymes: Wide range of enzymes present in plasma are proteins.

Most plasma proteins are synthesized in the liver; but immunoglobulins are produced by the reticuloendothelial system (RES), the liver and the plasma cells of the bone marrow; and enzymes are released from various organs.

Plasma proteins can be broadly classified into two groups:

a) Those, including albumin, which are synthesized by the liver, and

b) The immunoglobulins, which are produced by plasma cells of the bone marrow and lymphatic tissue, usually as part of an immune response.

Since it is the proteins that impart most of the colloid osmotic pressure within the blood vessels, their amount must remain within a narrow range – the biological reference interval. For total proteins it is 6.6 – 8.7 gm/dL. Normally, the albumin and globulins exist in a ratio of 2:1, but during an acute immune response, or when there is abnormal production of globulins, this ratio reduces or even reverses.

As mentioned above, many of these proteins also serve as carriers for the various nutrients and hormones within the blood stream. Some of them are enumerated below:

1. Albumin binds to divalent and trivalent cations like cuprous (Cu^{2+}) and ferric (Fe^{3+}). It also is a carrier of heme, bilirubin and biliverdin. It transports a few water-insoluble substances like non-essential fatty acids (NEFA) and steroids, as well.

2. Ceruloplasmin binds Cu^{2+}.

3. Transferrin carries Fe^{3+}.

4. Thyroid-binding globulin (TBG), as the name suggests, binds to thyroxine (T4) as well as tri-iodothyronine (T3).

5. Cortisol-binding globulin (CBG), similarly, transports cortisol.

6. Sex hormone binding globulins (SHBG) are transporters of the sex hormones, e.g. androgens (like testosterone), oestrogens (like oestradiol), etc.

We have described the basic structure of an immunoglobulin in connection with the blood group antibodies. Immunoglobulin G (molecular weight 160,000) is the major immunoglobulin (Ig) produced by the plasma cells and makes up to 70-75% of the total immunoglobulins (Igs). 65% of this is extravascular and the remainder is mainly present in the plasma. Its major function is neutralisation of toxins in tissue spaces. Hence these antibodies are produced in response to most bacteria and viruses. IgG1 and IgG2 subclasses cross the placenta by an active transport process dependent on the FC (constant fragment) binding. IgG1 is the principal IgG to cross the placenta and protect the neonates for the first three months of postnatal life.

Immunoglobulin M is the most primitive and least specialized immunoglobulin and the only immunoglobulin that a neonate synthesizes. It accounts for 5-10% of the total circulating immunoglobulins. IgM as a membrane receptor molecule is monomeric, but most of the serum IgM is pentameric and has a molecular weight of 900,000, the monomers being connected to each other by a joining fragment called the “J chain”.

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IgM is secreted in the first or “primary” response to an antigen by the B lymphocytes. The heavy chains of the IgM surface receptor molecules, then, are modified in situ to IgG or IgA heavy chains but the variable regions remain unchanged. As the B lymphocytes mature into plasma cells a second exposure to the antigen causes a larger “secondary” response, now of IgG secretion.

IgM continues to be synthesized against antigens confined to the blood, e.g. RBC surface antigens and tropical parasites.

Immunoglobulin A (molecular weight 160,000) makes up 10-15% of the serum Ig. IgA is monomeric, but 10-15% of IgA in serum will be polymeric. A more important form of IgA is called secretory IgA, which is found in tears, saliva, sweat, milk, colostrum and gastrointestinal and bronchial secretions. This secretory IgA is dimeric and has a molecular weight of 380,000, the two monomers being linked by a J chain. In addition, it has a secretory fragment. It is synthesized mainly by the plasma cells in the mucous membranes of the gut and bronchi, and in the ductules of the lactating breast.

**Biomedical aspects**

IgA can activate complement by the alternate pathway. Its presence in Colostrum and milk probably protects the neonate from intestinal infections.

Immunoglobulin D accounts for less than 1% of serum Igs. It is a monomer of molecular weight 184,000. Its primary function is unknown.

Immunoglobulin E, a monomer of molecular weight 188,000, is so rapidly and firmly bound to mast cells that only trace amounts are normally present in the serum. Many IgE molecules are attached to mast cell surfaces. Each molecule may be a different antibody produced by a different variable region; the IgE is attached through binding sites on the FC chains.

When antigen (allergen) crosslinks two of the attached IgE molecules, the mast cells is stimulated to release histamine and other vasoactive amines. These vasoactive amines are responsible for the vascular permeability and smooth muscle contraction occurring in such allergic reactions as hay fever, asthma, urticaria and eczema. Measurement of total circulating IgE level is of value in the early detection of allergy in infants and as a measure for predicting future atopic manifestations.

When we estimate the concentration of a single class of immunoglobulin, we are evaluating a polyclonal mixture of antibodies that are “idiotypes” – that is the product of many different clones of plasma cells, each clone producing immunoglobulin molecules with a different variable region. Benign or malignant proliferation can be of one such clone (where there is production of high concentration of a single idiotype – a monoclonal antibody) or of a few clones together so that the increased concentration of antibodies is oligoclonal.

Polyclonal increases of immunoglobulins are the normal response to infection.

Monoclonal increases in immunoglobulins occur when a single clone of plasma cells is permitted to multiply. These monoclonal immunoglobulins are also called ‘paraproteins’ and are detected as a single spike on electrophoresis.
Overproduction of any one type of immunoglobulin results in a decreased synthesis of the remaining immunoglobulins and, often, leaves the patient susceptible to infections.

**Homeostasis**

Homeostasis is a universal process (or an array of processes) by which all living systems maintain themselves within certain viable settings. The human body, too, needs to maintain its internal environment in a state conducive to healthy existence. The term was coined in 1932 by Walter Cannon from the Greek homo (same, like) and stasis (to stand, posture). 60% of the total body weight is comprised of water, which is distributed in two main compartments – the intracellular (70%) and the extracellular (30%); the latter is further subdivided into interstitial fluid (21%) and plasma (79%).

![Composition of Plasma](image1)

![Composition of Intercellular fluid](image2)

**Fig. 22: Composition of Plasma**  **Fig. 23: Composition of Intercellular fluid**

The composition of all body fluids varies dynamically in health and diseases. Maintaining the water in these fluids along with their other constituents is called homeostasis, i.e. maintainances of the nearly constant conditions in the milieu interior. It is achieved by an array of dynamic reactions and processes occurring in various organ systems of the body. For instance, the kidneys maintain a constant ionic concentration, the lungs send oxygen to all the body cells via the RBC in the circulation; the gastrointestinal tract provides the nutrients (having absorbed them from the food ingested by the person). It is a beautiful example of commendable team work.

The capillary wall, which separates the plasma from the interstitial fluid, is freely permeable to water and electrolytes, but restricts the flow of proteins. The result is that whereas ions and low molecular weight molecules are similarly distributed in the extracellular fluid (ECF) and plasma, the concentration of protein is four to five times greater in plasma than in the interstitial fluid.

Plasma cations amount to about 150mmol/L, of which 140mmol/L are sodium and 4mmol/L are potassium. The most abundant plasma anions are chloride and bicarbonate, which have an average concentration of 100mmol/L and 25mmol/L, respectively.

In the intracellular fluid (ICF), the main cation is potassium with a concentration of 110 mmol/L. This is about 30 times as much as in the ECF. On the other hand, sodium and
chloride, which are abundant in ECF, have a concentration as low as 10mmol/L and 4mmol/L, respectively in ICF. Anions in this fluid include proteins, phosphate, and other substances, which cannot diffuse freely through the all membrane.

This concentration gradient of potassium and sodium across a membrane that is permeable to these ions is maintained by an ATP-dependent Na\(^+\)-K\(^+\) ATPase pump in this membrane (Fig. 24). Here, the energy derived from hydrolysis of an ATP molecule drives three sodium ions from the cell to its exterior. In turn two potassium ions enter the cell from outside. Thus the membrane maintains chemical and electrical potential gradients between the inside and outside of a cell. The exact mechanism by which sodium is extruded and potassium imbibed is an interesting example of the intricacies of cellular function. The channel protein (or carrier protein) in the membrane has three sites for binding of the sodium ion on its intracellular facet. When intracellular Na\(^+\) binds to these sites and phosphorylation of this protein occurs, the resultant conformational change exposes, to the exterior of the cell, the Na\(^+\)-binding sites from which the sodium ions are then released. At the same time, the two potassium-binding sites become receptive and are occupied by K\(^+\).

![](image)

**Fig. 24: Cell membrane – Na\(^+\), K\(^+\) ATPase pump**

The release of sodium from its binding sites initiates dephosphorylation and a reverse conformational change in the protein molecule. Thus, the K\(^+\)-binding sites now face the interior of the cell and release the K\(^+\) into the cytoplasm. This electrochemical gradient plays a role in nerve transmission, too.

The passage of water across the ECF / ICF barrier is determined by its osmotic pressure. This is contributed by all the solutes present in fluids. An increase in ECF osmolality draws water from the ICF into the ECF.

Within the ECF, the distribution of water between the intravascular and extravascular compartments is determined by the concentration of plasma proteins, particularly albumin, which retains water in the vascular bed. This is known as oncotic pressure and is countered by the hydrostatic pressure in the blood vessels, which drives water out of the capillaries. Typically, at the arterial end, the hydrostatic pressure exceeds the oncotic pressure resulting in a filtering out of water and small molecular weight substances. At the venous end, however, the oncotic pressure exceeds the hydrostatic pressure and fluid is drawn into the blood vessel.
**Biomedical aspects**

When there is a deficiency of protein as in malnutrition or excessive loss, albumin levels in the plasma are low; hence, the hydrostatic pressure exceeds the oncotic pressure at the arterial as well as venous ends of the capillary. As a result, water (and small molecular weight substances) ooze out of the vessels and are not reabsorbed, leading to accumulation of fluid in the interstitial compartment. This is known as oedema.

**The role of kidneys**

All the electrolytes and water in the body are maintained by the kidney by balancing the input (which may be from dietary or metabolic sources) and the output (which may be by metabolic consumption or through the various modes of excretion). They maintain the composition, osmolality and volume of ECF and also control the acid-base balance.

When there is a decrease in the extracellular fluid sodium or when there is decreased blood volume or low blood pressure (as may occur in circulatory insufficiency), there is stimulation of the centers for thirst and salt–appetite, whereby water and / or sodium are replenished.

The kidneys remove products of metabolism (urea, uric acid, creatinine) and retain valuable substances such as glucose, amino acids and proteins. Sometimes they also metabolize and remove drugs and toxins. The functional unit of the kidneys is a nephron of which there are one million in each kidney. A nephron is comprised of a glomerulus and excretory tubules. The glomerulus connects the circulatory system with the excretory tubules.

![Fig. 25: The nephron](image-url)
At the glomerulus, about 150 liters of plasma is filtered every day by the Bowman’s capsule which is a globular, basement cell–lined funnel that engulfs the glomerulus. This funnel transports the water and aqueous wastes (including metabolic wastes, excess mineral wastes and toxins) into the proximal convoluted tubules.

The volume and composition of the glomerular filtrate change during its passage through the various parts of the nephron. Mainly, there is reabsorption of sodium, chloride, glucose,
amino acids, phosphates and other anions, where as $H^+$ and $K^+$ are transported from the interstitial fluid to the distal convoluted tubules.

The two hormone systems that help the kidney in this regulation are:

1. **Vasopressin** - This hormone, also known as antidiuretic hormone (ADH), increases the amount of water retained by increasing its reabsorption from kidney tubules and promoting thirst.

2. **The Rennin – Angiotensin-Aldosterone System**

Renal artery hypotension (as may occur in fluid loss) or hypoxia or decreased sodium delivery to the renal tubules stimulate the release of rennin from the juxta-glomerular apparatus. This promotes conversion of circulating Angiotensinogen to Angiotensin I. The vascular endothelial cells especially in the lungs, have an enzyme – Angiotensin Converting Enzyme (+ ATPase ACE) – which cleaves two amino acids from the Angiotensin I to yield Angiotensin II. Angiotensin II acts on the adrenal cortex as well as on the pituitary. On the adrenal cortex, its action stimulates the release of aldosterone (a mineralocorticoid) which acts on the Na+ - K system in the living cells of the collecting ducts of the kidneys, resulting in sodium and water reabsorption and extrusion of K+. On the pituitary, its effect causes secretion of ADH, which acts at the same site to increase water reabsorption and on the CNS sites for promoting thirst. Both these actions result in an increase in the blood volume and, thereby, an inhibition of this very chain of reactions – the negative feedback mechanism.

**Biomedical aspects**

a) **Maintenance of the homeostatic water and electrolyte balance**

1. Each substance reabsorbed in the renal tubules has a specific transport maximum, beyond which concentration it will not be reabsorbed. This is the basis of estimating the presence or absence of glucose in urine during management of diabetics. (The glomerular maximum, or $G_{max}$, for glucose is 180 mg/dL; if the level of glucose in the plasma exceeds this concentration, it will be filtered through the glomerulus and excreted in the urine.)

2. Deficiency of vasopressin causes Diabetes Insipidus, which is characterized by excretion of large amounts of dilute urine coupled with insatiable thirst.

In contrast, an excessive secretion of the hormone takes place following major surgery or trauma – the syndrome of inappropriate secretion of ADH (SIADH). This leads to water retention.

b) **Endocrine causes of hypertension**

1. A raised rennin (due to anomalous JG apparatus) can cause high blood pressure.

2. Increased aldosterone secretion can occur due to a single adrenal tumour, or, more commonly, a phaeochromocytoma (a catecholamine secreting tumour).

An increase in either of these hormones causes increased water and sodium retention, thereby increasing the vascular hydrostatic pressure, which is clinically manifest as hypertension.
The control of acid – base balance

Why do we need to maintain a balance between the acidity and alkalinity of our body fluids? The answer is simple.

1. Proteins contain many negatively charged and basic groups within their structure. A change in the hydrogen ion concentration in the milieu will automatically disrupt these charges and this can interfere with the function of this protein. e.g. hemoglobin and its oxygen-carrying capacity.

2. Some proteins that are enzymes function normally within a very narrow range of pH. For most enzymes the optimum pH is that of plasma (7.35 – 7.45).

[pH of any fluid is the negative logarithm of the hydrogen ion concentration of that fluid.]

Since enzymes are a part of the functional units of every system in the body, change in pH of plasma (and thereby of interstitial and intracellular fluids) alters all body functions, sometimes with grave results.

Substantial quantities of inorganic and organic acids are generated from the dissolving of metabolically produced CO₂ and the metabolism of sulphur-containing amino acids and phosphorus-containing compounds. Acids derived from CO₂ sources are termed volatile as they get converted to CO₂, which is excreted through the lungs during respiration; those derived from other sources are called non-volatile acids (lactic acid and keto-acids). These cannot be removed by the lungs and depend on the kidneys for their excretion.

When these modes of excretion are interfered with, there is either an increase in the hydrogen ion concentration, [H⁺], in the blood (acidity, low pH), or a decrease in these ions (alkalinity, high pH).

To combat the minor changes that occur on a daily basis as a result of diet as well as metabolic processes, the body has various buffer systems. These are:

- Bicarbonate buffers
- Phosphate buffers
- Proteins

In co-operation with these systems, the lungs and kidneys help regulate the pH.

The bicarbonate buffer are simple chemicals. Proteins, on the other hand, are complex molecules. It is the carbonyl (-COOH) and the amide (-NH₂) groups present on these molecules that allow them to behave as buffers. Of the proteins, haemoglobin is a major buffer.

a) The bicarbonate buffer

This buffer system is unique as it remains in equilibrium with the atmospheric air. This enables it to be many times more efficient than other closed buffer systems.

As shown in Fig. 17 representing the Bohr effect, carbon dioxide equilibrates with H₂O in plasma through a very slow, non enzymatic reaction. Hence there is very little carbonic acid present in the plasma.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \quad \text{[in tissue]}
\]
Thus the plasma pH is determined by the ratio between the concentrations of plasma bicarbonate (the ‘base’ component of the buffer) and the dissolved CO₂ (the ‘acid’ component). At the normal plasma pCO₂ of 5.3 Kpa (1.2mmol/L), the erythrocytes and renal tubular cells maintain the bicarbonate content of the plasma at its mean value of 24mmol/L.

When the acidity of blood increases (as in excessive exercise), these react with the bicarbonate to release CO₂. As the concentration of CO₂ increases more of it is pushed across the membrane lining the alveoli (due to the high concentration gradient). In addition, an increase in the pCO₂ and decrease in pH act directly on the respiratory center in the brain stem (medulla oblangata) causing an increase in ventilation.

A typical bicarbonate buffer system consists of a mixture of carbonic acid (H₂CO₃) and sodium bicarbonate (NaHCO₃) in the same solution. In our body fluids we also have smaller quantities of potassium bicarbonate [KHCO₃], calcium bicarbonate [Ca(HCO₃)₂] and magnesium bicarbonate [Mg(HCO₃)₂] which serve the same function as NaHCO₃. In the ECF, where NaHCO₃ is abundant, these lesser bicarbonates are a minor support system for the main buffer; but in the ICF, where there is little NaHCO₃, KHCO₃ and Mg(HCO₃)₂ take over the buffering functions. In the body fluids, carbonic acid is rarely present as such due to its constant dissociation into water and CO₂ – a normally slow process. The result is a high concentration of dissolved CO₂, but only a weak concentration of acid.

When an acid, like HCL, is added to this buffer system, the following reaction ensues:

\[
\text{HCL} + \text{NaHCO}_3 \rightarrow \text{H}_2\text{CO}_3 + \text{NaCl}
\]

Thus the strong hydrochloric acid [HCl] is converted into the weak carbonic acid. Thus, the HCl lowers the pH only slightly.

Similarly, when a base like sodium hydroxide [NaOH] is added to this system, the following reaction takes place:

\[
\text{NaOH} + \text{H}_2\text{CO}_3 \rightarrow \text{NaHCO}_3 + \text{H}_2\text{O}
\]

Thus, there is a loss of carbonic acid from the solution but since this is a weak acid, the hydrogen ion concentration (and pH) barely varies.

**Dissociation of carbonic acid**

All acids are ionised to a certain extent and the percentage of ionisation is called the degree of dissociation.

\[
\text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^-
\]

This is a reversible dynamic reaction and its shift to the right or left depends on the constant changes in our milieu (interior mostly, but exterior, too).

When the equation is in equilibrium, the product of the concentration of the two ions on the right have a constant numerical relation to the concentration of the acid itself., i.e.

\[
\frac{[\text{H}^+] \times [\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = K_1 \text{ (a constant)} \quad \{\text{equation 1}\}
\]
If you consider the complete reaction where CO$_2$ and H$_2$O combine to form carbonic acid and then the carbonic acid dissociates, we have

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^-$$

Here, too,

$$[\text{H}^+] \times [\text{HCO}_3^-] = K \quad \text{(equation 2)}$$

$$[\text{CO}_2]$$

It has been experimentally observed that $K_1 \approx 1000K$. Equation 2 can also be expressed as follows:

$$[\text{H}^+] = K \times \frac{[\text{CO}_2]}{[\text{HCO}_3^-]}$$

Taking the logarithm of the two sides, we have:

$$\log [\text{H}^+] = \log K + \log \frac{[\text{CO}_2]}{[\text{HCO}_3^-]}$$

Hence,

$$\frac{1}{\log [\text{H}^+]} = \frac{1}{\log K} + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}$$

Further,

$$-\log [\text{H}^+] = -\log K + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}$$

We have already mentioned that $-\log [\text{H}^+]$ is the pH. Similarly, $-\log K = \text{pK}$.

Replacing these two values in the above reaction, we have

$$\text{pH} = \text{pK} + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}$$

The pK is constant for every type of solution. For the bicarbonate buffer system, pK = 6.1. Hence, the equation becomes:

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}$$

This is called the *Handersen-Hasselbach equation*, which can be used to calculate pH.

As compared to the other buffer systems of the body, the bicarbonate is a weak buffer. Yet, it is a very important system, mainly because each of the two elements of this system can be regulated; the CO$_2$ is regulated by ventilation and the bicarbonate by the kidneys.

**b) The Phosphate Buffer system**

This is similar to the bicarbonate buffer system in its reactions, but its constituents are different; they are NaH$_2$PO$_4$ and Na$_2$HPO$_4$. Normally these dissociate in such a way that NaH$_2$PO$_4$ behaves as a weak acid, whereas Na$_2$HPO$_4$ behaves like a weak base.

When a strong acid like HCl is added, the resultant reaction is:

$$\text{HCl} + \text{Na}_2\text{HPO}_4 \rightarrow \text{NaH}_2\text{PO}_4 + \text{NaCl}$$
Thus the hydrochloric acid is removed and an additional quantity of NaH$_2$PO$_4$ is formed. Since NaH$_2$PO$_4$ is only weakly acid, the deleterious effects of the strong acid HCl get tempered almost totally.

On the other hand, when a strong base like sodium hydroxide (NaOH) is added, the reaction is:

\[ \text{NaOH} + \text{NaH}_2\text{PO}_4 \rightleftharpoons \text{Na}_2\text{HPO}_4 + \text{H}_2\text{O} \]

Here, the strong base NaOH is replaced by a weak base Na$_2$HPO$_4$.

The phosphate buffer has a pK of 6.8, which is close to the pH of the body fluids, i.e. 7.4. Hence, in the body, it functions near to its maximum capacity. Yet, since its concentration in the ECF is only one-sixth of the bicarbonate buffer, it serves as a lesser buffer than the bicarbonate system. It is only in the kidney tubules that the phosphate buffer system has an upper hand due to:

a) its higher concentration in the tubules, which makes it the major system there, and
b) the pH of the tubular fluids which are nearer its pK of 6.8 and hence it functions to its maximum.

c) The protein buffer system

The most plentiful buffer in the body is the protein. Hydrogen ions can diffuse to some extent across the membrane, carbon dioxide diffuses readily and even the bicarbonate ion can diffuse to some extent. These form a direct link between the ECF and the ICF, so much so that changes in the pH of the ECF are reflected in the ICF. Thus, all the buffer systems in the cell help to buffer the ECF as well. In fact, about 75% of the chemical buffering power in the body lies within the cells and most of this results from the intracellular proteins.

Proteins are amino acids bound by peptide linkages. Some of the amino acids have a free acidic radical, -COOH, which dissociates to give the –COO⁻ negative radical and the H⁺ ion. Also, some of them have free basic radicals, -NH$_3$OH, which dissociate into NH$_3$⁺ and the OH⁻ ions. Thus, proteins have both acidic and basic buffering systems. Also, their pK is close to 7.4, which makes them very efficient in the human body.

The three buffer systems have been described individually, but in actual physiological systems they work together, for the H⁺ is common to them all. Hence when the [H⁺] of one system is disturbed, it effects the other two as well, as denoted by the equation given below:

\[ [\text{H}^+] = \frac{K_1 \times \text{HA}_1^+}{A_1} = \frac{K_2 \times \text{HA}_2^+}{A_2} = \frac{K_3 \times \text{HA}_3^+}{A_3} \]

This is called the iso hydric principle where all the buffer systems interact to maintain a constant [H⁺]. In fact, these buffer systems actually buffer each other.

d) The respiratory regulation of acid-base balance is by the Bohr effect described in the section on oxygen carriage by hemoglobin. Its efficacy as a regulator results from the fact that the [H⁺] has a direct effect on the respiratory centre in the brain stem (medulla oblangata). Because of the ability of the respiratory centre to respond to [H⁺] and because changes in the alveolar ventilation, in turn, alter the [H⁺] in the body fluids, the respiratory system acts as a typical feedback regulatory system for the control of [H⁺].
Unfortunately, respiratory control cannot return the [H⁺] to normal. Hence, it serves as a support to the chemical buffer systems, especially for the bicarbonate buffers.

e) Intracellular buffering
Normally, the H⁺ enter the cell in exchange for K⁺. When extracellular buffering of H⁺ occurs, intracellular K⁺ will increase. Also, when bicarbonate ions (HCO₃⁻) increase in the extracellular fluids, H⁺ from the cells buffer this increase of HCO₃⁻. And, in exchange for H⁺, K⁺ are driven into the cell, again increasing intracellular K⁺ and decreasing plasma K⁺.

Hence, acidemia (high [H⁺] in plasma) may be associated with hyperkalemia, and alkalinemia (low [H⁺] in plasma) may be associated with hypokalemia.

f) Renal regulation of [H⁺]
This is principally affected through the handling of the bicarbonate ion in the renal tubules. The proximal tubules, the distal tubules and the collecting ducts, all secrete [H⁺] into the tubular fluids. Within the tubular cell, the CO₂ (under the influence of the enzyme carbonic anhydrase) combines with water to give carbonic acid, which then dissociates into the H⁺ and the HCO₃⁻. This H⁺ is secreted through the tubular membrane into the tubule. Thus, the greater the CO₂ in the ECF, the greater is the rate of H⁺ secretion.

Hence, any factor that increases the CO₂ concentration in the ECF (such as decreased respiration or increased metabolic rate) increases the H⁺ secretion from the tubules. Conversely, any factor that decreases the CO₂ (as in increased respiration or decreased metabolic rate) also decreases the rate of H⁺ secretion. This secretion is maximal (85%) in the proximal tubules, where a three-fold gradient may be achieved across the membrane; but the collecting ducts can continue secreting H⁺ till the pH of the tubular fluid (urine) falls to 4.5.

In exchange of these H⁺ ions secreted, Na⁺ ions are reabsorbed. Usually, HCO₃⁻ are being constantly filtered through the glomerular basement membrane into the glomerular filtrate and this is usually in combination with Na⁺ ions and, to a lesser extent, with other positive ions of the ECF. From the tubules sodium ions are reabsorbed into the lining cells in exchange for H⁺ ions extruded. So the H⁺ ions now combine with HCO₃⁻ ions to form carbonic acid, which dissociates into CO₂ and H₂O. All the CO₂ then diffuses through the epithelial lining cells into the peritubular fluids while the water passes into the urine.

Hence, if enough H⁺ ions are available, HCO₃⁻ ions are completely removed from the tubules, so that practically none is excreted in the urine.

Renal correction of alkalosis
When the ratio of the bicarbonate ions to the dissolved CO₂ molecules increases, pH rises into the alkalosis range (>7.4). This causes an increased filtration of bicarbonate into the tubules and hence the ratio of bicarbonates in the tubules to the H⁺ ions (secreted from the cells) increases. So the fine balance of H⁺ ions and HCO₃⁻ ions in the tubules is altered. There is much more bicarbonate and since it cannot be reabsorbed without combination with H⁺ ions, it passes out with the urine. These ions then carry Na⁺ ions (or other positive ions) into the urine. The tubular cells then excrete these Na⁺ ions to maintain the intracellular K⁺ and H⁺ concentrations.
ions) along with them. This resultant removal of sodium bicarbonate from the ECF bicarbonate buffer system results in an increase in the $H^+$ ion concentration and, thereby, a shift of pH towards the acidic range, thus correcting the alkalosis.

**Renal correction of acidosis**

In acidosis the ratio of $CO_2$ to $HCO_3^-$ ions in ECF increases which is exactly the opposite of alkalosis. Hence, the $H^+$ ion secretion rises to a level far greater than the rate of $HCO_3^-$ ion filtration into the tubules. As a result, there are an excess of $H^+$ ions secreted which have no $HCO_3^-$ ions to react with, in the tubules. At the same time that the $H^+$ ion is secreted from a tubular cell, a $HCO_3^-$ ion is produced in that cell and also a $Na^+$ ion is absorbed from the tubule. These ions ($Na^+$ and $HCO_3^-$) diffuse together in the peritubular fluid. Thus, the net effect of secreting excess $H^+$ ions into the tubules is an increase in the quantity of sodium bicarbonate (NaHCO$_3$) in the ECF bicarbonate buffer. As a result, there is a decrease in the $H^+$ ion concentration and a rise in the pH towards alkalinity. Thus, acidosis gets corrected.

**Biomedical aspects - Acid-Base disorders**

1. **Acidosis** is a much more common disorder than alkalosis and it can sub classified into either respiratory or metabolic acidosis.
**Respiratory acidosis** occurs most often in lung disease and results from decreased ventilation e.g. chronic obstructive lung disease (COLD), severe asthma and even hypoxia where the low \( pO_2 \) is accompanied by a high \( pCO_2 \).

**Metabolic acidosis** results from the excessive production, or inefficient metabolism or excretion of non-volatile acids. A classic example is diabetic ketoacidosis, which is a result of unopposed lipolysis resulting in a accumulation of acetyl CoA and its metabolites (\( \alpha \)-hydroxybutyrate and acetoacetate) in plasma. Extreme physical exercise leads to increased muscle metabolism with resultant accumulation of lactate. Normally, this would be cleared away soon after cessation of exercise. But when very large quantities of lactate are generated as a consequence of hypoxia, acidosis results. In certain instances like shock, it becomes life threatening. Sometimes, in renal disease (e.g. glomerulonephritis), renal failure or slow renal perfusion (as in shock), the excretion of non volatile acids is impeded, again resulting in metabolic acidosis.

Severe diarrhoea or surgical drainage after bowel surgery leads to excessive loss of bicarbonate (as may also occur in impaired renal re-absorption of bicarbonate) causes metabolic acidosis.

2. **Alkalolis** is rarer than acidosis. Hyperventillation, as may occur in exercise, anxiety attack, fever or even pregnancy can cause mild respiratory alkalosis.

**Metabolic alkalosis** is often associated with abnormally low potassium concentration in plasma, as a result of cellular buffering. Thus alkalosis can cause hypokalemia, and primary hypokalemia may lead to alkalosis.

Severe metabolic alkalosis can result from severe vomiting due to massive loss of hydrogen ions with the vomitus. This also happens when a patient is on nasogastric suction.

Rarely, it can occur when too much bicarbonate is given intravenously, as during resuscitation after cardiac arrest.

**General biomedical aspects of plasma constituents**

In addition to these very important electrolytes and proteins, and their regulation, it is imperative to mention, here, that every type of molecule that is present in the body is also present in the plasma of the blood in a regulated amount. Its concentration in the blood depends on its concentration required in its effector tissue or the tissue where it is synthesized. Thus, each of these substances is present in the plasma in a specific concentration or range of concentration that is viable to the organ system as a whole. This range of concentration is known as the biological reference interval (BRI).

There are a multitude of feedback mechanisms, which strive to keep all the constituents of the plasma within their biological reference interval. Any anomaly in any of the organ systems and the feedback mechanisms anywhere in the body is reflected in an increase or decrease in the concentration of the constituent involved.

Hence, if the nephrons of the kidneys are not functioning optimally, the wastes that they extrude in the urine accumulate in the plasma, e.g.
Urea: BRI: 11-45 mg/dL
Creatinine: BRI: 0.6-1.3 mg/dL

Even electrolytes show an altered concentration; and the degree of alteration is proportional to the degree of malfunction. Similarly, malfunction of the liver can manifest in a variety of ways:

If the synthetic pathways are at fault, the alteration will be reflected in the concentration of:
- Total proteins: BRI: 6.6-8.7 gm/dL and
- Albumin: BRI: 3.5-5.0 gm/dL

Also, other proteins will show an altered concentration in the plasma. If the metabolic pathways (congenital or acquired) are altered, there is an alteration in:
- Total Lipids: BRI: 400-1000 mg/dL
- Cholesterol: BRI: 120-190 mg/dL and
- Triglycerides: BRI: 35-160 mg/dL, and

the various lipoproteins and apolipoproteins.

And if it is the hepatocytes (cells of the ‘hepatos’ or liver) are damaged, then the enzymes that are released from these cells will rise. These include:
- Aspartate aminotransferase: BRI: 0-42 IU/L
- Alanine aminotransferase: BRI: 0-60 IU/L
- Alkaline phosphatase: BRI: 39-117 IU/L in adults
  117-390 IU/L in children
- Gamma glutamyl transferase: BRI: 0-64 IU/L

Again the degree of alteration will depend on the degree and site (within the liver) of damage or malfunction. The alkaline phosphatase in the plasma can also come from the bone; hence, any anomaly in that tissue is reflected in the level of this enzyme. During the latter half of pregnancy too, it is raised as it is secreted by the placenta.

Another example is the pancreas which has two types of cells – the α and the β cells. The α cells are responsible for the manufacture of the pancreatic enzymes – amylase, lipase, etc. - whereas the β cells synthesize insulin which regulates glucose metabolism. When the pancreas gets inflamed, it is mostly the pancreatic enzymes amylase and lipase (other sources of amylase and lipase include saliva and intestines) that are raised due to an increased release of these substances from the injured α cells.
- Amylase: BRI: 5-100 U/L (depends on method used for analysis)
- Lipase: BRI: <190 U/L (depends on method used for analysis)

On the other hand, the β cells are affected mostly by chronic inflammation, fibrosis, etc and this results in a decrease in the secretion of insulin (which is reflected in its concentration in the plasma) and, thereby, an increase in the concentration of glucose in the plasma. The concentration of glucose in the plasma is also dependent on the stage of feeding, so that the BRI’s for the fasting state and the postprandial state (2 hours after a meal) are different.
- Glucose fasting: BRI: 70-100 mg/dL
- Glucose post-prandial: BRI: 90-140 mg/dL

When the feeding status is unknown, it is called a ‘random glucose’ level.
- Glucose random: BRI: 70-160 mg/dL
When any sort of muscle is damaged or dystrophied, the enzyme from these cells shows an increased level in the plasma. For e.g. when the cardiac muscle is damaged as in myocardial infarction, creatinine kinase (CK) and its cardio-specific iso-enzyme, the MB (muscle brain) fraction, are found to be raised. This enzyme (CK) is also raised after any sort of trauma to muscles, be it mild as in case of intramuscular injections or even mild exercise, or severe as in road-side accidents or other type of crush-injury (say, in a factory); in this case it is the MM (muscle muscle) fraction that is mostly elevated.

(The CK-MM fraction is calculated by subtracting the MB fraction from the total CK.)

These are all substances that are present in macro-quantities. There are, now, many known chemical constituents of the plasma, which, though they are present only in micro-quantities, are important indicators of the state of function of various systems. Examples are some elements, vitamins, hormones, etc.

As an example, we may look at the assessment of the red blood cell synthesis or function. This is achieved not only by estimating the hemoglobin concentration and other red blood cell parameters, as is done in the hematological laboratory, but also by estimating each of the factors that contributes to hemoglobin synthesis and red cell maturation, e.g.

<table>
<thead>
<tr>
<th>Substance</th>
<th>BRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>50-150 µg/dL</td>
</tr>
<tr>
<td>Ferritin (iron storage molecule)</td>
<td>20-300 ng/mL in males and 15-120 ng/mL in females</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>220-960 pg/mL</td>
</tr>
<tr>
<td>Folic acid</td>
<td>3-17 ng/dL</td>
</tr>
</tbody>
</table>

Yet another function of the plasma is to transport drugs that are ingested, injected or infused from the site of administration to the site of action. Depending on the mode of administration, the concentration achieved in the plasma varies and, hence, so does the amount delivered to the target tissue. To assess its efficacy, the concentration is measured in the laboratory and the dose adjusted accordingly. Their concentration, too, is in micro-quantities. Some examples are anti-epileptics, anti-asthmatics, anti-arrythmics, other cardio-active drugs and antibiotics.

Thus, it is evident that measuring the concentration of a substance in the blood can be used to assess the status of the organ system in which it is synthesized or utilised, or, if it is a drug, its efficacy in the target tissue. And that is precisely what the clinical biochemist does, in conjunction with the clinician, towards proper diagnoses and management of patients.

Suggested Reading